(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

D47

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 November 2001 (22.11.2001)

PCT

(10) International Publication Number
WO 01/88126 A2

(51) International Patent Classification[7]: C12N 15/12, 15/62, C07K 14/705, C12Q 1/68, G01N 33/68, A61K 31/7088, 39/395

(21) International Application Number: PCT/EP01/05383

(22) International Filing Date: 11 May 2001 (11.05.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/204,145 15 May 2000 (15.05.2000) US
60/250,505 4 December 2000 (04.12.2000) US

(71) Applicant *(for all designated States except US)*: **BAYER AKTIENGESELLSCHAFT** [DE/DE]; 51368 Leverkusen (DE).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **RAMAKRISHNAN, Shyam** [IN/US]; 76 Euston Road, Apt. #10, Brighton, MA 02135 (US).

(74) Common Representative: **BAYER AKTIENGESELLSCHAFT**; 51368 Leverkusen (DE).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/88126 A2

(54) Title: REGULATION OF HUMAN α1A?ADRENERGIC RECEPTOR-LIKE G PROTEIN-COUPLED RECEPTOR

(57) Abstract: Reagents which regulate human α_{1a}adrenergic receptor-like GPCR and reagents which bind to human α_{1a} adrenergic receptor-like GPCR gene products can play a role in preventing, ameliorating, or correcting dysfunctions or diseases including, but not limited to, infections such as bacterial, fungal, protozoan, and viral infections, particularly those caused by HIV viruses, pain, obesity cancers, anorexia, bulimia, asthma, Parkinson's diseases, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, angina pectoris, myocardial infarction, ulcers, asthma, allergies, multiple sclerosis, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, several mental retardation, and dyskinesias, such as Huntington's disease and Tourett's syndrome.

REGULATION OF HUMAN α_{1A} ADRENERGIC RECEPTOR-LIKE G PROTEIN-COUPLED RECEPTOR

TECHNICAL FIELD OF THE INVENTION

The invention relates to the area of G-protein coupled receptors. More particularly, it relates to the area of human α_{1a} adrenergic receptor-like G protein-coupled receptors and their regulation.

BACKGROUND OF THE INVENTION

G-Protein Coupled Receptors

Many medically significant biological processes are mediated by signal transduction pathways that involve G-proteins (Lefkowitz, *Nature 351*, 353-354, 1991). The family of G-protein coupled receptors (GPCR) includes receptors for hormones, neurotransmitters, growth factors, and viruses. Specific examples of GPCRs include receptors for such diverse agents as dopamine, calcitonin, adrenergic hormones, endothelin, cAMP, adenosine, acetylcholine, serotonin, histamine, thrombin, kinin, follicle stimulating hormone, opsins, endothelial differentiation gene-1, rhodopsins, odorants, cytomegalovirus, G-proteins themselves, effector proteins such as phospholipase C, adenyl cyclase, and phosphodiesterase, and actuator proteins such as protein kinase A and protein kinase C.

GPCRs possess seven conserved membrane-spanning domains connecting at least eight divergent hydrophilic loops. GPCRs (also known as 7TM receptors) have been characterized as including these seven conserved hydrophobic stretches of about 20 to 30 amino acids, connecting at least eight divergent hydrophilic loops. Most GPCRs have single conserved cysteine residues in each of the first two extracellular loops, which form disulfide bonds that are believed to stabilize functional protein

structure. The seven transmembrane regions are designated as TM1, TM2, TM3, TM4, TM5, TM6, and TM7. TM3 has been implicated in signal transduction.

Phosphorylation and lipidation (palmitylation or farnesylation) of cysteine residues can influence signal transduction of some GPCRs. Most GPCRs contain potential phosphorylation sites within the third cytoplasmic loop and/or the carboxy terminus. For several GPCRs, such as the β-adrenergic receptor, phosphorylation by protein kinase A and/or specific receptor kinases mediates receptor desensitization.

For some receptors, the ligand binding sites of GPCRs are believed to comprise hydrophilic sockets formed by several GPCR transmembrane domains. The hydrophilic sockets are surrounded by hydrophobic residues of the GPCRs. The hydrophilic side of each GPCR transmembrane helix is postulated to face inward and form a polar ligand binding site. TM3 has been implicated in several GPCRs as having a ligand binding site, such as the TM3 aspartate residue. TM5 serines, a TM6 asparagine, and TM6 or TM7 phenylalanines or tyrosines also are implicated in ligand binding.

GPCRs are coupled inside the cell by heterotrimeric G-proteins to various intracellular enzymes, ion channels, and transporters (*see* Johnson *et al.*, *Endoc. Rev. 10*, 317-331, 1989). Different G-protein alpha-subunits preferentially stimulate particular effectors to modulate various biological functions in a cell. Phosphorylation of cytoplasmic residues of GPCRs is an important mechanism for the regulation of some GPCRs. For example, in one form of signal transduction, the effect of hormone binding is the activation inside the cell of the enzyme, adenylate cyclase. Enzyme activation by hormones is dependent on the presence of the nucleotide GTP. GTP also influences hormone binding. A G-protein connects the hormone receptor to adenylate cyclase. G-protein exchanges GTP for bound GDP when activated by a hormone receptor. The GTP-carrying form then binds to activated adenylate cyclase. Hydrolysis of GTP to GDP, catalyzed by the G-protein itself, returns the G-protein to its basal, inactive form. Thus, the G-protein serves a

dual role, as an intermediate that relays the signal from receptor to effector, and as a clock that controls the duration of the signal.

Over the past 15 years, nearly 350 therapeutic agents targeting GPCRs receptors have been successfully introduced onto the market. This indicates that these receptors have an established, proven history as therapeutic targets. Clearly, there is an on-going need for identification and characterization of further GPCRs which can play a role in preventing, ameliorating, or correcting dysfunctions or diseases including, but not limited to, infections such as bacterial, fungal, protozoan, and viral infections, particularly those caused by HIV viruses, pain, cancers, anorexia, bulimia, asthma, Parkinson's diseases, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, several mental retardation, and dyskinesias, such as Huntington's disease and Tourett's syndrome.

Adrenergic Receptors

Human adrenergic receptors are integral membrane proteins which have been classified into two broad classes, the alpha and the beta adrenergic receptors (see U.S. Patent 5,922,722). Both types mediate the action of the peripheral sympathetic nervous system upon binding of catecholamines, norepinephrine and epinephrine.

Norepinephrine is produced by adrenergic nerve endings, while epinephrine is produced by the adrenal medulla. The binding affinity of adrenergic receptors for these compounds forms one basis of the classification: alpha receptors bind norepinephrine more strongly than epinephrine and much more strongly than the synthetic compound isoproterenol. The binding affinity of these hormones is reversed for the beta receptors. In many tissues, the functional responses, such as

smooth muscle contraction, induced by alpha receptor activation are opposed to responses induced by beta receptor binding.

Subsequently, the functional distinction between alpha and beta receptors was further highlighted and refined by the pharmacological characterization of these receptors from various animal and tissue sources. As a result, alpha and beta adrenergic receptors were further subdivided into α_1, α_2, α_1, and α_2 subtypes. Functional differences between α_1 and α_2 receptors have been recognized, and compounds which exhibit selective binding between these two subtypes have been developed. Thus, in WO 92/0073, the selective ability of the R(+) enantiomer of terazosin to selectively bind to adrenergic receptors of the α_1 subtype was reported. The α_1/ α_2 selectivity of this compound was disclosed as being significant because agonist stimulation of the α_2 receptors was said to inhibit secretion of epinephrine and norepinephrine, while antagonism of the α_2 receptor was said to increase secretion of these hormones. Thus, the use of non-selective α-adrenergic blockers, such as phenoxybenzamine and phentolamine, is limited by their α_2 adrenergic receptor mediated induction of increased plasma catecholamine concentration and the attendant physiological sequelae (increased heart rate and smooth muscle contraction).

For a general background on the α-adrenergic receptors, see Ruffolo, α-adrenoreceptors: Molecular Biology, Biochemistry and Pharmacology (Progress in Basic and Clinical Pharmacology series, Karger, 1991), wherein the basis of α_1 /α_2 subclassification, the molecular biology, signal transduction (G-protein interaction and location of the significant site for this and ligand binding activity away from the 3'-terminus of alpha adrenergic receptors), agonist structure-activity relationships, receptor functions, and therapeutic applications for compounds exhibiting α-adrenergic receptor affinity is discussed.

The cloning, sequencing, and expression of alpha receptor subtypes from animal tissues has led to the subclassification of the α_1 receptors into α_{1a} (Lomasney *et al.*,

J. Biol. Chem. 266, 6365-369, 1991); rat α_{1a} (Bruno *et al.*, *BBRC 179*, 1485-90, 1991), human α_{1a}, α_{1b} (Cotecchia *et al.*, *Proc. Natl. Acad. Sci. 85*, 7159-63, 1988), hamster α_{1b} (Libert *et al.*, *Science* 1989), dog α_{1b} (Ramarao *et al.*, *J. Biol. Chem. 267*, 21936-945, 1992), and human α_{1b}. Most recently, in a study using bovine brain, a new α_{1c} subtype was proposed (Schwinn *et al.*, *J. Biol. Chem. 265*, 8183-89, 1990). Hirasawa *et al.* (*BBRC 195*, 902-09, 1993) described the cloning, functional expression. and tissue distribution of a human α_{1c} adrenergic receptor. Hoehe *et al.*, *Human Mol. Genetics 1*(5) 349, 1992) noted the existence of a two-allele Pstl restriction fragment polymorphism in the α_{1c} adrenergic receptor gene. Another study suggests that there may even be an α_{1d} receptor subtypes (see Perez *et al.*, *Mol. Pharm. 40*, 876-83, 1992). Each α_1 receptor subtype exhibits its own pharmacologic and tissue specificities. Schwirm and coworkers noted that the cloned bovine α_{1c} receptor exhibited pharmacological properties proposed for the α_{1a} subtype. Nonetheless, based on its non-expression in tissues where the α_{1a} subtype is expressed and its sensitivity to chloroethylclonidine, the receptor was given a new designation.

The differences in the α-adrenergic receptor subtypes have relevance in patho-physiologic conditions. For example, benign prostatic hyperplasia, also known as benign prostatic hypertrophy or BPH, is an illness typically affecting men over fifty years of age, increasing in severity with increasing age. The symptoms of the condition include, but are not limited to, increased difficulty in urination and sexual dysfunction. These symptoms are induced by enlargement (hyperplasia) of the prostate gland. As the prostate increases in size, it impinges on free-flow of fluids through the male urethra. Concomitantly, the increased noradrenergic innervation of the enlarged prostate leads to an increased adrenergic tone of the bladder neck and urethra, further restricting the flow of urine through the urethra.

The male hormone 5α-dihydrotestosterone has been identified as the principal culprit in benign prostatic hyperplasia. The continual production of 5α-dihydrotestosterone

by the male testes induces incremental growth of the prostate gland throughout the life of the male. In many men beyond the age of about fifty years, this enlarged gland begins to obstruct the urethra with the pathologic symptoms noted above. However, as may be appreciated from the lengthy development of the syndrome, its reversal also is not immediate. In the interim, those males suffering with BPH continue to suffer.

In response to this problem, one solution is to identify pharmaceutically active compounds which complement slower-acting therapeutics by providing acute relief. Agents which induce relaxation of the urethral smooth muscle, by binding to α_1 adrenergic receptors, thus reducing the increased adrenergic tone due to the disease, would be good candidates for this activity. Thus, one such agent is alfuzosin, which is reported in EP 0 204597 to induce urination in cases of prostatic hyperplasia. Likewise, in WO 92/0073, the selective ability of the R(+) enantiomer of terazosin to bind to adrenergic receptors of the α_1 subtype was reported. In addition, in WO 92/161213, combinations of 5α-reductase inhibitory compounds and α_1 adrenergic receptor blockers (terazosin, doxazosin, prazosin, bunazosin, indoramin, alfuzosin) were disclosed. However, no information as to the α_{1a}, α_{1b}, α_{1c}, subtype specificity of these compounds was provided. Current therapy for BPH uses existing non-selective α_1 antagonists such as prazosin (Minipress, Pfizer), Terazosin (Hytrin, Abbott) or doxazosin mesylate (Cardura, Pfizer). These non-selective antagonists suffer from side effects related to antagonism of the α_{1a} and α_{1b} receptors in the peripheral vasculature, *e.g.*, orthostatic hypotension and syncope.

Typically, identification of active compounds is accomplished through use of animal tissues known to be enriched in adrenergic receptors. Thus, rat tissues have been used to screen for potential adrenergic receptor antagonists. However, because of species variability, compounds which appear active in animal tissue may not be active or sufficiently selective in humans. This results in substantial waste of time and effort, particularly where high volume compound screening programs are employed. There is also the danger that compounds, which might be highly effective

in humans, would be missed because of their absence of appreciable affinity for the heterologous animal receptors. In this regard, it has been noted that even single amino acid changes between the sequence of biologically active proteins in one species may give rise to substantial pharmacological differences. Thus, Fong *et al.* (*J. Biol. Chem, 267*, 25668-71, 1992) showed that there are 22 divergent amino acid residues between the sequence of the human neurokinin-1 receptor and the homologous rat receptor. They further showed, in studies with mutant receptors, that substitution of only two amino acid residues was both necessary and sufficient to reproduce the rat receptor's antagonist binding affinity in the human receptor. Oksenberg *et al.* (*Nature 360*, 161-63, 1992) showed that a single amino-acid difference confers major pharmacological variation between the human and the rodent 5-hydroxytryptamine receptors. Likewise, Kuhse *et al. (Neuron 5*, 867-873, 1990) showed that a single amino-acid exchange alters the pharmacology of the neonatal rat glycine receptor subunit. This difficulty and unpredictability has resulted in a need for a compound screen which will identify compounds that will be active in humans. Thus, there is a need in the art to identify human α_1-like adrenergic receptors whose activity can be regulated to provide therapeutic effects.

SUMMARY OF THE INVENTION

It is an object of the invention to provide reagents and methods of regulating a human G protein-coupled receptor. This and other objects of the invention are provided by one or more of the embodiments described below.

One embodiment of the invention is a α_{1a} adrenergic receptor-like GPCR polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO. 3; and

the amino acid sequence shown in SEQ ID NO. 3.

Yet another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a α_{1a} adrenergic receptor-like GPCR polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO. 3; and

the amino acid sequence shown in SEQ ID NO. 3.

Binding between the test compound and the α_{1a} adrenergic receptor-like GPCR polypeptide is detected. A test compound which binds to the α_{1a} adrenergic receptor-like GPCR polypeptide is thereby identified as a potential agent for decreasing extracellular matrix degradation.

Another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a polynucleotide encoding a α_{1a} adrenergic receptor-like GPCR polypeptide, wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of:

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO. 2; and

the nucleotide sequence shown in SEQ ID NO. 2.

Binding of the test compound to the polynucleotide is detected. A test compound which binds to the polynucleotide is identified as a potential agent for decreasing extracellular matrix degradation. The agent can work by decreasing the amount of the

α_{1a} adrenergic receptor-like GPCR through interacting with the α_{1a} adrenergic receptor-like GPCR mRNA.

Another embodiment of the invention is a method of screening for agents which regulate extracellular matrix degradation. A test compound is contacted with a α_{1a} adrenergic receptor-like GPCR polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO. 3; and

the amino acid sequence shown in SEQ ID NO. 3.

A α_{1a} adrenergic receptor-like GPCR activity of the polypeptide is detected. A test compound which increases α_{1a} adrenergic receptor-like GPCR activity of the polypeptide relative to α_{1a} adrenergic receptor-like GPCR activity in the absence of the test compound is thereby identified as a potential agent for increasing extracellular matrix degradation. A test compound which decreases α_{1a} adrenergic receptor-like GPCR activity of the polypeptide relative to α_{1a} adrenergic receptor-like GPCR activity in the absence of the test compound is thereby identified as a potential agent for decreasing extracellular matrix degradation.

Even another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a α_{1a} adrenergic receptor-like GPCR product of a polynucleotide which comprises a nucleotide sequence selected from the group consisting of:

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO. 2; and

the nucleotide sequence shown in SEQ ID NO. 2.

Binding of the test compound to the α_{1a} adrenergic receptor-like GPCR polypeptide product is detected. A test compound which binds to the α_{1a} adrenergic receptor-like GPCR product is thereby identified as a potential agent for decreasing extracellular matrix degradation.

Still another embodiment of the invention is a method of reducing extracellular matrix degradation. A cell is contacted with a reagent which specifically binds to a polynucleotide encoding a α_{1a} adrenergic receptor-like GPCR polypeptide or the product encoded by the polynucleotide, wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of:

nucleotide sequences which are at least about 50% identical to the nucleotide sequence shown in SEQ ID NO. 2; and

the nucleotide sequence shown in SEQ ID NO. 2.

α_{1a} adrenergic receptor-like GPCR activity in the cell is thereby decreased.

The invention thus provides a human α_{1a} adrenergic receptor-like GPCR which can be used to identify test compounds which may act as agonists or antagonists at the receptor site. Human α_{1a} adrenergic receptor-like GPCR and fragments thereof also are useful in raising specific antibodies which can block the receptor and effectively prevent ligand binding.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the human genomic DNA on chromosome 22 comprising the CDS of an α_{1a} adrenergic receptor.

Fig. 2 shows the DNA-sequence encoding a α_{1a} adrenergic receptor-like GPCR polypeptide (CDS).

Fig. 3 shows the amino acid sequence of a α_{1a} adrenergic receptor-like GPCR polypeptide encoded by the DNA sequence of Fig. 2.

Fig. 4 shows the amino acid sequence of *Oryctolagus cuniculus* protein polypeptide.

Fig. 5 shows the promotor region and the first 118 nucleotides of the DNA-sequence of Fig. 1.

Two modules are found in the promotor region:

A. EBOX_E2FF_01

1. Promoter module: EBOX_E2FF_01 (consists of two transacting binding domains, EBOX and E2F)

2. The module is involved in the activation of the Epstein-Barr virus pol promoter.

3. Reference: Liu C. et al., J. Virol. 70, 2545-2555, 1996 (MEDLINE: 8642684)

B. GATA_AP1F_01

1. Promoter module: GATA_AP1F_01 (consists of two transacting binding domains, GATA and AP1F)

2. The module is required for the constitutive expression of the IL-5 gene in adult T-cell leukimia cells.

3. Reference: Yamagata T. et al., Mol. Cell. Biol. 17, 4272-4281, 1997 (MEDLINE: 9234684)

Fig. 6 shows the alignment of α_{1a} adrenergic receptor-like GPCR of Fig. 3 with SwissProt Accession No. O02824 of Fig. 4 (expectation value 4e-25).

Fig. 7 shows the BLASTX - alignment of α_{1a} adrenergic receptor-like GPCR of Fig. 3 with SwissProt Accession No. O02824 of Fig. 4 (expectation value 0.013).

Fig. 8 shows the relative expression of the α_{1a} adrenergic receptor-like GPCR in different human tissues and cell lines as determined by RT-PCR.

Fig. 9 shows the relative expression of the α_{1a} adrenergic receptor-like GPCR in different human tissues as determined by RT-PCR using phage libraries or cDNA as template

Fig. 10 shows the relative expression of the α_{1a} adrenergic receptor-like GPCR in different human tissues as determined by Taqman analysis

Fig. 11 shows the relative expression of the α_{1a} adrenergic receptor-like GPCR in different human tissues relevant for cardiovascular diseases as determined by Taqman analysis

Fig. 12 shows the relative expression of the α_{1a} adrenergic receptor-like GPCR in different human tissues relevant for peripheral or central nervous system diseases as determined by Taqman analysis

DETAILED DESCRIPTION OF THE INVENTION

The invention relates to an isolated polynucleotide encoding a α_{1a} adrenergic receptor-like GPCR polypeptide and being selected from the group consisting of:

a) a polynucleotide encoding a α_{1a} adrenergic receptor-like GPCR polypeptide comprising an amino acid sequence selected from the group consisting of:
amino acid sequences which are at least about 50% identical to
the amino acid sequence shown in SEQ ID NO. 3; and
the amino acid sequence shown in SEQ ID NO. 3.

b) a polynucleotide comprising the sequence of SEQ ID NO. 2;

c) a polynucleotide which hybridizes under stringent conditions to a polynucleotide specified in (a) and (b);

d) a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code; and

e) a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a) to (d).

Furthermore, it has been discovered by the present applicant that a α_{1a} adrenergic receptor-like GPCR, particularly a human α_{1a} adrenergic receptor-like GPCR, can be

used in therapeutic methods to treat disorders such as bacterial, fungal, protozoan, and viral infections, particularly those caused by HIV viruses, pain, cancers, anorexia, bulimia, asthma, Parkinson's diseases, obesity, acute heart failure, hypotension, hypertension, urinary retention, osteoporosis, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, several mental retardation, and dyskinesias, such as Huntington's disease and Tourett's syndrome. Human α1a adrenergic receptor-like GPCR also can be used to screen for α_{1a} adrenergic receptor- like GPCR agonists and antagonists.

Human α_{1a} adrenergic receptor-like GPCR is 30% identical over 216 amino acids to the *Oryctolagus cuniculus* protein having SwissProt Accession No. O02824 (SEQ ID NO.4) and annotated as an α_{1a} adrenergic receptor (Fig. 4). Human α_{1a} adrenergic receptor-like GPCR also is 29% identical over 74 amino acids to O02824. Human α_{1a} adrenergic receptor-like GPCR therefore is expected to have an α_{1a} adrenergic receptor- like function and to be useful for the same purposes as previously identified α_{1a} adrenergic receptors. Human α_{1a} adrenergic receptor-like GPCR has transmembrane regions from amino acids 36-53, 71-89, 108-125, 144-161, 189-210, 395-417, and 428-450.

Polypeptides

Human α_{1a} adrenergic receptor-like GPCR polypeptides according to the invention comprise at least 10, 20, 30, 40, 50, 75, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450, or 500 contiguous amino acids selected from the amino acid sequence shown in SEQ ID NO. 3 or a biologically active variant thereof, as defined below. A human α_{1a} adrenergic receptor-like GPCR polypeptide of the invention therefore can be a portion of a human α_{1a} adrenergic receptor-like GPCR protein, a full-length human α_{1a} adrenergic receptor-like GPCR protein, or a fusion protein comprising all or a portion of a human α_{1a} adrenergic receptor-like GPCR protein.

Biologically Active Variants

GPCR polypeptide variants which are biologically active, *i.e.*, retain the ability to bind a ligand to produce a biological effect, such as cyclic AMP formation, mobilization of intracellular calcium, or phosphoinositide metabolism, also are GPCR polypeptides. Preferably, naturally or non-naturally occurring GPCR polypeptide variants have amino acid sequences which are at least about 50, preferably about 75, 90, 96, or 98% identical to an amino acid sequence shown in SEQ ID NO. 3 or a fragment thereof. Percent identity between a putative GPCR polypeptide variant and an amino acid sequence of SEQ ID NO. 3 is determined using the Blast2 alignment program (using Blosum62, Expect 10, standard genetic codes).

Variations in percent identity can be due, for example, to amino acid substitutions, insertions, or deletions. Amino acid substitutions are defined as one for one amino acid replacements. They are conservative in nature when the substituted amino acid has similar structural and/or chemical properties. Examples of conservative replacements are substitution of a leucine with an isoleucine or valine, an aspartate with a glutamate, or a threonine with a serine.

Amino acid insertions or deletions are changes to or within an amino acid sequence. They typically fall in the range of about 1 to 5 amino acids. Guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing biological or immunological activity of a GPCR polypeptide can be found using computer programs well known in the art, such as DNASTAR software. Whether an amino acid change results in a biologically active GPCR polypeptide can readily be determined by assaying for binding to a ligand or by conducting a functional assay, as described for example, in the specific Examples, below.

Fusion Proteins

Fusion proteins are useful for generating antibodies against GPCR polypeptide amino acid sequences and for use in various assay systems. For example, fusion proteins can be used to identify proteins which interact with portions of a GPCR polypeptide. Protein affinity chromatography or library-based assays for protein-protein interactions, such as the yeast two-hybrid or phage display systems, can be used for this purpose. Such methods are well known in the art and also can be used as drug screens.

A human α_{1a} adrenergic receptor-like GPCR fusion protein comprises two polypeptide segments fused together by means of a peptide bond. The first polypeptide segment comprises at least 10, 20, 30, 40, 50, 75, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450, or 500 contiguous amino acids of SEQ ID NO. 3 or a biologically active variant thereof, such as those described above. The first polypeptide segment also can comprise full-length human α_{1a} adrenergic receptor-like GPCR.

The second polypeptide segment can be a full-length protein or a protein fragment. Proteins commonly used in fusion protein construction include β-galactosidase, β-glucuronidase, green fluorescent protein (GFP), autofluorescent proteins, including blue fluorescent protein (BFP), glutathione-S-transferase (GST), luciferase, horseradish peroxidase (HRP), and chloramphenicol acetyltransferase (CAT). Additionally, epitope tags are used in fusion protein constructions, including histidine (His) tags, FLAG tags, influenza hemagglutinin (HA) tags, Myc tags, VSV-G tags, and thioredoxin (Trx) tags. Other fusion constructions can include maltose binding protein (MBP), S-tag, Lex a DNA binding domain (DBD) fusions, GAL4 DNA binding domain fusions, and herpes simplex virus (HSV) BP16 protein fusions. A fusion protein also can be engineered to contain a cleavage site located between the GPCR polypeptide-encoding sequence and the heterologous protein sequence, so

that the GPCR polypeptide can be cleaved and purified away from the heterologous moiety.

A fusion protein can be synthesized chemically, as is known in the art. Preferably, a fusion protein is produced by covalently linking two polypeptide segments or by standard procedures in the art of molecular biology. Recombinant DNA methods can be used to prepare fusion proteins, for example, by making a DNA construct which comprises coding sequences selected from the complement of SEQ ID NO. 2 in proper reading frame with nucleotides encoding the second polypeptide segment and expressing the DNA construct in a host cell, as is known in the art. Many kits for constructing fusion proteins are available from companies such as Promega Corporation (Madison, WI), Stratagene (La Jolla, CA), CLONTECH (Mountain View, CA), Santa Cruz Biotechnology (Santa Cruz, CA), MBL International Corporation (MIC; Watertown, MA), and Quantum Biotechnologies (Montreal, Canada; 1-888-DNA-KITS).

Identification of Species Homologs

Species homologs of human α_{1a} adrenergic receptor-like GPCR polypeptides can be obtained using polynucleotides of the invention (described below) to make suitable probes or primers for screening cDNA expression libraries from other species, such as mice, monkeys, or yeast, identifying cDNAs which encode homologs of GPCR polypeptide, and expressing the cDNAs as is known in the art.

Polynucleotides

A human α_{1a} adrenergic receptor-like GPCR polynucleotide can be single- or double-stranded and comprises a coding sequence or the complement of a coding sequence for a human α_{1a} adrenergic receptor-like GPCR polypeptide. A coding sequence for human α_{1a} adrenergic receptor-like GPCR is shown in SEQ ID NO. 2. The coding sequence obeys the classical KOZAK rule for the transcription start site. The coding

sequence of SEQ ID NO.1 comprises this coding sequence and also contains a promoter module (EBOX_E2FF_01), which consists of two trans-acting binding domains, EBOX and E2F. The module is involved in the activation of the Epstein-Barr virus pol promoter. See Liu *et al., J. Virol. 70*, 2545-55, 1996. SEQ ID NO.1 also contains a promoter module (GATA_AP1F_01), which consists of two trans-acting binding domains, GATA and AP1F. The module is required for the constitutive expression of the IL-5 gene in adult T-cell leukemia cells. See Yamagata *et al., Mol. Cell. Biol. 17*, 4272-81, 1997.

Degenerate nucleotide sequences encoding human α_{1a} adrenergic receptor-like GPCR polypeptides, as well as homologous nucleotide sequences which are at least about 50, preferably about 75, 90, 96, or 98% identical to the nucleotide sequences shown in SEQ ID NO.1 or 3 also are human α_{1a} adrenergic receptor-like GPCR polynucleotides. Percent sequence identity between the sequences of two polynucleotides is determined using computer programs such as ALIGN which employ the FASTA algorithm, using an affine gap search with a gap open penalty of -12 and a gap extension penalty of -2. Complementary DNA (cDNA) molecules, species homologs, and variants of GPCR polynucleotides which encode biologically active GPCR polypeptides also are GPCR polynucleotides.

Identification of Polynucleotide Variants and Homologs

Variants and homologs of the polynucleotides described above also are human α_{1a} adrenergic receptor-like GPCR polynucleotides. Typically, homologous polynucleotide sequences can be identified by hybridization of candidate polynucleotides to known human α_{1a} adrenergic receptor-like GPCR polynucleotides under stringent conditions, as is known in the art. For example, using the following wash conditions--2X SSC (0.3 M NaCl, 0.03 M sodium citrate, pH 7.0), 0.1% SDS, room temperature twice, 30 minutes each; then 2X SSC, 0.1% SDS, 50 °C once, 30 minutes; then 2X SSC, room temperature twice, 10 minutes each--homologous sequences can be identified which contain at most about 25-30% basepair

mismatches. More preferably, homologous nucleic acid strands contain 15-25% basepair mismatches, even more preferably 5-15% basepair mismatches.

Species homologs of the polynucleotides disclosed herein also can be identified by making suitable probes or primers and screening cDNA expression libraries from other species, such as mice, monkeys, or yeast. Human variants of human α_{1a} adrenergic receptor-like GPCR polynucleotides can be identified, for example, by screening human cDNA expression libraries. It is well known that the T_m of a double-stranded DNA decreases by 1-1.5°C with every 1% decrease in homology (Bonner *et al.*, *J. Mol. Biol. 81*, 123 (1973). Variants of human α_{1a} adrenergic receptor-like GPCR polynucleotides or homologous polynucleotides of other species can therefore be identified by hybridizing a putative homologous polynucleotide with a polynucleotide having a nucleotide sequence of SEQ ID NO.1 or 3 or the complement thereof to form a test hybrid. The melting temperature of the test hybrid is compared with the melting temperature of a hybrid comprising polynucleotides having perfectly complementary nucleotide sequences, and the number or percent of basepair mismatches within the test hybrid is calculated.

Nucleotide sequences which hybridize to human α_{1a} adrenergic receptor-like GPCR polynucleotides or their complements following stringent hybridization and/or wash conditions also are human α_{1a} adrenergic receptor-like GPCR polynucleotides. Stringent wash conditions are well known and understood in the art and are disclosed, for example, in Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, 2d ed., 1989, at pages 9.50-9.51.

Typically, for stringent hybridization conditions a combination of temperature and salt concentration should be chosen that is approximately 12-20 °C below the calculated T_m of the hybrid under study. The T_m of a hybrid between a GPCR polynucleotide having a nucleotide sequence shown in SEQ ID NO.1 or 3 or the complement thereof and a polynucleotide sequence which is at least about 50, preferably about 75, 90, 96, or 98% identical to one of those nucleotide sequences

can be calculated, for example, using the equation of Bolton and McCarthy, *Proc. Natl. Acad. Sci. U.S.A. 48*, 1390 (1962):

$T_m = 81.5°C - 16.6(\log_{10}[Na^+]) + 0.41(\%G + C) - 0.63(\%formamide) - 600/l)$,

where l = the length of the hybrid in basepairs.

Stringent wash conditions include, for example, 4X SSC at 65°C, or 50% formamide, 4X SSC at 42°C, or 0.5X SSC, 0.1% SDS at 65°C. Highly stringent wash conditions include, for example, 0.2X SSC at 65°C.

Preparation of Polynucleotides

A naturally occurring polynucleotide can be isolated free of other cellular components such as membrane components, proteins, and lipids. Polynucleotides can be made by a cell and isolated using standard nucleic acid purification techniques, or synthesized using an amplification technique, such as the polymerase chain reaction (PCR), or by using an automatic synthesizer. Methods for isolating polynucleotides are routine and are known in the art. Any such technique for obtaining a polynucleotide can be used to obtain isolated polynucleotides. For example, restriction enzymes and probes can be used to isolate polynucleotide fragments which comprises human α_{1a} adrenergic receptor- like GPCR nucleotide sequences. Isolated polynucleotides are in preparations which are free or at least 70, 80, or 90% free of other molecules.

Human α_{1a} adrenergic receptor-like GPCR cDNA molecules can be made with standard molecular biology techniques, using human α_{1a} adrenergic receptor-like GPCR mRNA as a template. cDNA molecules can thereafter be replicated using molecular biology techniques known in the art and disclosed in manuals such as Sambrook *et al.* (1989). An amplification technique, such as PCR, can be used to obtain additional copies of polynucleotides of the invention, using either human genomic DNA or cDNA as a template.

Alternatively, synthetic chemistry techniques can be used to synthesize human α_{1a} adrenergic receptor-like GPCR polynucleotides. The degeneracy of the genetic code allows alternate nucleotide sequences to be synthesized which will encode a polypeptide having, for example, an amino acid sequence shown in SEQ ID NO. 3 or a biologically active variant thereof.

Extending Polynucleotides

Various PCR-based methods can be used to extend the nucleic acid sequences encoding the disclosed portions of human α_{1a} adrenergic receptor-like GPCR to detect upstream sequences such as promoters and regulatory elements. For example, restriction-site PCR uses universal primers to retrieve unknown sequence adjacent to a known locus (Sarkar, *PCR Methods Applic. 2*, 318-322, 1993). Genomic DNA is first amplified in the presence of a primer to a linker sequence and a primer specific to the known region. The amplified sequences are then subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

Inverse PCR also can be used to amplify or extend sequences using divergent primers based on a known region (Triglia *et al.*, *Nucleic Acids Res. 16*, 8186, 1988). Primers can be designed using commercially available software, such as OLIGO 4.06 Primer Analysis software (National Biosciences Inc., Plymouth, Minn.), to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68-72°C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

Another method which can be used is capture PCR, which involves PCR amplification of DNA fragments adjacent to a known sequence in human and yeast

artificial chromosome DNA (Lagerstrom *et al.*, *PCR Methods Applic. 1*, 111-119, 1991). In this method, multiple restriction enzyme digestions and ligations also can be used to place an engineered double-stranded sequence into an unknown fragment of the DNA molecule before performing PCR.

Another method which can be used to retrieve unknown sequences is that of Parker *et al.*, *Nucleic Acids Res. 19*, 3055-3060, 1991). Additionally, PCR, nested primers, and PROMOTERFINDER libraries (CLONTECH, Palo Alto, Calif.) can be used to walk genomic DNA (CLONTECH, Palo Alto, Calif.). This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. Randomly-primed libraries are preferable, in that they will contain more sequences which contain the 5' regions of genes. Use of a randomly primed library may be especially preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries can be useful for extension of sequence into 5' non-transcribed regulatory regions.

Commercially available capillary electrophoresis systems can be used to analyze the size or confirm the nucleotide sequence of PCR or sequencing products. For example, capillary sequencing can employ flowable polymers for electrophoretic separation, four different fluorescent dyes (one for each nucleotide) which are laser activated, and detection of the emitted wavelengths by a charge coupled device camera. Output/light intensity can be converted to electrical signal using appropriate software (*e.g.* GENOTYPER and Sequence NAVIGATOR, Perkin Elmer), and the entire process from loading of samples to computer analysis and electronic data display can be computer controlled. Capillary electrophoresis is especially preferable for the sequencing of small pieces of DNA which might be present in limited amounts in a particular sample.

Obtaining GPCR Polypeptides

Human α_{1a} adrenergic receptor-like GPCR polypeptides can be obtained, for example, by purification from human cells, by expression of polynucleotides, or by direct chemical synthesis.

Protein Purification

Human α_{1a} adrenergic receptor-like GPCR polypeptides can be purified from any human cell which expresses the receptor, including host cells which have been transfected with human α_{1a} adrenergic receptor-like GPCR polynucleotides. A purified polypeptide is separated from other compounds which normally associate with the polypeptide in the cell, such as certain proteins, carbohydrates, or lipids, using methods well-known in the art. Such methods include, but are not limited to, size exclusion chromatography, ammonium sulfate fractionation, ion exchange chromatography, affinity chromatography, and preparative gel electrophoresis.

A human α_{1a} adrenergic receptor-like GPCR polypeptide can be conveniently isolated as a complex with its associated G protein, as described in the specific examples, below. A preparation of purified human α_{1a} adrenergic receptor-like GPCR polypeptides is at least 80% pure; preferably, the preparations are 90%, 95%, or 99% pure. Purity of the preparations can be assessed by any means known in the art, such as SDS-polyacrylamide gel electrophoresis.

Expression of Polynucleotides

To express a human α_{1a} adrenergic receptor-like GPCR polypeptide, a human α_{1a} adrenergic receptor-like GPCR polynucleotide can be inserted into an expression vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods which are well known to those skilled in the art can be used to construct expression vectors containing sequences encoding human

α_{1a} adrenergic receptor- like GPCR polypeptides and appropriate transcriptional and translational control elements. These methods include *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Such techniques are described, for example, in Sambrook *et al.* (1989) and in Ausubel *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, N.Y., 1989.

A variety of expression vector/host systems can be utilized to contain and express sequences encoding a human α_{1a} adrenergic receptor-like GPCR polypeptide. These include, but are not limited to, microorganisms, such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors, insect cell systems infected with virus expression vectors (*e.g.*, baculovirus), plant cell systems transformed with virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (*e.g.*, Ti or pBR322 plasmids), or animal cell systems.

The control elements or regulatory sequences are those non-translated regions of the vector -- enhancers, promoters, 5' and 3' untranslated regions -- which interact with host cellular proteins to carry out transcription and translation. Such elements can vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, can be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the BLUESCRIPT phagemid (Stratagene, LaJolla, Calif.) or pSPORT1 plasmid (Life Technologies) and the like can be used. The baculovirus polyhedrin promoter can be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (*e.g.*, heat shock, RUBISCO, and storage protein genes) or from plant viruses (*e.g.*, viral promoters or leader sequences) can be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell line that contains multiple copies of a

nucleotide sequence encoding a GPCR polypeptide, vectors based on SV40 or EBV can be used with an appropriate selectable marker.

Bacterial and Yeast Expression Systems

In bacterial systems, a number of expression vectors can be selected depending upon the use intended for the GPCR polypeptide. For example, when a large quantity of a GPCR polypeptide is needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified can be used. Such vectors include, but are not limited to, multifunctional *E. coli* cloning and expression vectors such as BLUESCRIPT (Stratagene). In a BLUESCRIPT vector, a sequence encoding the GPCR polypeptide can be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of β-galactosidase so that a hybrid protein is produced. pIN vectors (Van Heeke & Schuster, *J. Biol. Chem. 264*, 5503-5509, 1989) or pGEX vectors (Promega, Madison, Wis.) also can be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems can be designed to include heparin, thrombin, or factor Xa protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

In the yeast *Saccharomyces cerevisiae*, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH can be used. For reviews, see Ausubel *et al.* (1989) and Grant *et al.*, *Methods Enzymol. 153*, 516-544, 1987.

Plant and Insect Expression Systems

If plant expression vectors are used, the expression of sequences encoding GPCR polypeptides can be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV can be used alone or in combination with the omega leader sequence from TMV (Takamatsu, *EMBO J. 6*, 307-311, 1987). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters can be used (Coruzzi *et al.*, *EMBO J. 3*, 1671-1680, 1984; Broglie *et al.*, *Science 224*, 838-843, 1984; Winter *et al.*, *Results Probl. Cell Differ. 17*, 85-105, 1991). These constructs can be introduced into plant cells by direct DNA transformation or by pathogen-mediated transfection. Such techniques are described in a number of generally available reviews (*e.g.*, Hobbs or Murray, in MCGRAW HILL YEARBOOK OF SCIENCE AND TECHNOLOGY, McGraw Hill, New York, N.Y., pp. 191-196, 1992).

An insect system also can be used to express a GPCR polypeptide. For example, in one such system *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia* larvae. Sequences encoding GPCR polypeptides can be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of GPCR polypeptides will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses can then be used to infect *S. frugiperda cells* or *Trichoplusia* larvae in which GPCR polypeptides can be expressed (Engelhard *et al.*, *Proc. Nat. Acad. Sci. 91*, 3224-3227, 1994).

Mammalian Expression Systems

A number of viral-based expression systems can be used to express GPCR polypeptides in mammalian host cells. For example, if an adenovirus is used as an expression vector, sequences encoding GPCR polypeptides can be ligated into an

adenovirus transcription/translation complex comprising the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome can be used to obtain a viable virus which is capable of expressing a GPCR polypeptide in infected host cells (Logan & Shenk, *Proc. Natl. Acad. Sci. 81*, 3655-3659, 1984). If desired, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, can be used to increase expression in mammalian host cells.

Human artificial chromosomes (HACs) also can be used to deliver larger fragments of DNA than can be contained and expressed in a plasmid. HACs of 6M to 10M are constructed and delivered to cells via conventional delivery methods (*e.g.*, liposomes, polycationic amino polymers, or vesicles).

Specific initiation signals also can be used to achieve more efficient translation of sequences encoding GPCR polypeptides. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding a GPCR polypeptide, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals (including the ATG initiation codon) should be provided. The initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons can be of various origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used (see Scharf *et al.*, *Results Probl. Cell Differ. 20*, 125-162, 1994).

Host Cells

A host cell strain can be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed GPCR polypeptide in the desired fashion. Such modifications of the polypeptide include, but are not limited to,

acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the polypeptide also can be used to facilitate correct insertion, folding and/or function. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (*e.g.*, CHO, HeLa, MDCK, HEK293, and WI38), are available from the American Type Culture Collection (ATCC; 10801 University Boulevard, Manassas, VA 20110-2209) and can be chosen to ensure the correct modification and processing of the foreign protein.

Stable expression is preferred for long-term, high-yield production of recombinant proteins. For example, cell lines which stably express GPCR polypeptides can be transformed using expression vectors which can contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells can be allowed to grow for 1-2 days in an enriched medium before they are switched to a selective medium. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced GPCR sequences. Resistant clones of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell type. See, for example, ANIMAL CELL CULTURE, R.I. Freshney, ed., 1986.

Any number of selection systems can be used to recover transformed cell lines.

These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler *et al.*, *Cell 11*, 223-32, 1977) and adenine phosphoribosyltransferase (Lowy *et al.*, *Cell 22*, 817-23, 1980) genes which can be employed in *tk*$^-$ or *aprt*$^-$ cells, respectively. Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate (Wigler *et al.*, *Proc. Natl. Acad. Sci. 77*, 3567-70, 1980), *npt* confers resistance to the aminoglycosides, neomycin and G-418 (Colbere-Garapin *et al.*, *J. Mol. Biol. 150*, 1-14, 1981), and *als* and *pat* confer resistance to chlorsulfuron and phosphinotricin

acetyltransferase, respectively (Murray, 1992, *supra*). Additional selectable genes have been described. For example, *trpB* allows cells to utilize indole in place of tryptophan, or *hisD*, which allows cells to utilize histinol in place of histidine (Hartman & Mulligan, *Proc. Natl. Acad. Sci. 85*, 8047-51, 1988). Visible markers such as anthocyanins, β-glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, can be used to identify transformants and to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes *et al.*, *Methods Mol. Biol. 55*, 121-131, 1995).

Detecting Expression of Polypeptides

Although the presence of marker gene expression suggests that the human α_{1a} adrenergic receptor-like GPCR polynucleotide is also present, its presence and expression may need to be confirmed. For example, if a sequence encoding a human α_{1a} adrenergic receptor-like GPCR polypeptide is inserted within a marker gene sequence, transformed cells containing sequences which encode a human α_{1a} adrenergic receptor-like GPCR polypeptide can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding a human α_{1a} adrenergic receptor-like GPCR polypeptide under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the GPCR polynucleotide.

Alternatively, host cells which contain a human α_{1a} adrenergic receptor-like GPCR polynucleotide and which express a human α_{1a} adrenergic receptor-like GPCR polypeptide can be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immunoassay techniques which include membrane, solution, or chip-based technologies for the detection and/or quantification of nucleic acid or protein. For example, the presence of a polynucleotide sequence encoding a human α_{1a} adrenergic receptor-like GPCR polypeptide can be detected by DNA-DNA or DNA-RNA hybridization or amplification

using probes or fragments or fragments of polynucleotides encoding a GPCR polypeptide. Nucleic acid amplification-based assays involve the use of oligonucleotides selected from sequences encoding a human α_{1a} adrenergic receptor-like GPCR polypeptide to detect transformants which contain a human α_{1a} adrenergic receptor-like GPCR polynucleotide.

A variety of protocols for detecting and measuring the expression of a human α_{1a} adrenergic receptor-like GPCR polypeptide, using either polyclonal or monoclonal antibodies specific for the polypeptide, are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay using monoclonal antibodies reactive to two non-interfering epitopes on a human α_{1a} adrenergic receptor-like GPCR polypeptide can be used, or a competitive binding assay can be employed. These and other assays are described in Hampton *et al.*, SEROLOGICAL METHODS: A LABORATORY MANUAL, APS Press, St. Paul, Minn., 1990) and Maddox *et al.*, *J. Exp. Med. 158*, 1211-1216, 1983).

A wide variety of labels and conjugation techniques are known by those skilled in the art and can be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding human α_{1a} adrenergic receptor-like GPCR polypeptides include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, sequences encoding a human α_{1a} adrenergic receptor-like GPCR polypeptide can be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and can be used to synthesize RNA probes *in vitro* by addition of labeled nucleotides and an appropriate RNA polymerase such as T7, T3, or SP6. These procedures can be conducted using a variety of commercially available kits (Amersham Pharmacia Biotech, Promega, and US Biochemical). Suitable reporter molecules or labels which can be used for ease of detection include radionuclides, enzymes, and

fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Expression and Purification of GPCR Polypeptides

Host cells transformed with nucleotide sequences encoding a human α_{1a} adrenergic receptor-like GPCR polypeptide can be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The polypeptide produced by a transformed cell can be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode human α_{1a} adrenergic receptor-like GPCR polypeptides can be designed to contain signal sequences which direct secretion of soluble polypeptides through a prokaryotic or eukaryotic cell membrane or which direct the membrane insertion of membrane-bound polypeptide.

As discussed above, other constructions can be used to join a sequence encoding a human α_{1a} adrenergic receptor-like GPCR polypeptide to a nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, Wash.). Inclusion of cleavable linker sequences such as those specific for Factor Xa or enterokinase (Invitrogen, San Diego, CA) between the purification domain and the human α_{1a} adrenergic receptor-like GPCR polypeptide also can be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing a human α_{1a} adrenergic receptor-like GPCR polypeptide and 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification by IMAC (immobilized metal ion affinity chromatography, as described in Porath *et al.*, *Prot. Exp. Purif. 3*, 263-281, 1992), while the enterokinase cleavage site provides a means for purifying

the polypeptide from the fusion protein. Vectors which contain fusion proteins are disclosed in Kroll *et al.*, *DNA Cell Biol. 12*, 441-453, 1993.

Chemical Synthesis

Sequences encoding a human α_{1a} adrenergic receptor-like GPCR polypeptide can be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers *et al.*, *Nucl. Acids Res. Symp. Ser.* 215-223, 1980; Horn *et al. Nucl. Acids Res. Symp. Ser.* 225-232, 1980). Alternatively, a GPCR polypeptide itself can be produced using chemical methods to synthesize its amino acid sequence, such as by direct peptide synthesis using solid-phase techniques (Merrifield, *J. Am. Chem. Soc. 85*, 2149-2154, 1963; Roberge *et al.*, *Science 269*, 202-204, 1995). Protein synthesis can be performed using manual techniques or by automation. Automated synthesis can be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Optionally, fragments of GPCR polypeptides can be separately synthesized and combined using chemical methods to produce a full-length molecule.

The newly synthesized peptide can be substantially purified by preparative high performance liquid chromatography (*e.g.*, Creighton, PROTEINS: STRUCTURES AND MOLECULAR PRINCIPLES, WH Freeman and Co., New York, N.Y., 1983). The composition of a synthetic human α_{1a} adrenergic receptor-like GPCR polypeptide can be confirmed by amino acid analysis or sequencing (*e.g.*, the Edman degradation procedure; *see* Creighton, *supra*). Additionally, any portion of the amino acid sequence of the human α_{1a} adrenergic receptor-like GPCR polypeptide can be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins to produce a variant polypeptide or a fusion protein.

Production of Altered Polypeptides

As will be understood by those of skill in the art, it may be advantageous to produce polypeptide-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce an RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

The nucleotide sequences disclosed herein can be engineered using methods generally known in the art to alter human α_{1a} adrenergic receptor-like GPCR polypeptide- encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the polypeptide or mRNA product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides can be used to engineer the nucleotide sequences. For example, site-directed mutagenesis can be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, introduce mutations, and so forth.

Antibodies

Any type of antibody known in the art can be generated to bind specifically to an epitope of a human α_{1a} adrenergic receptor-like GPCR polypeptide. "Antibody" as used herein includes intact immunoglobulin molecules, as well as fragments thereof, such as Fab, $F(ab')_2$, and Fv, which are capable of binding an epitope of a human α_{1a} adrenergic receptor-like GPCR polypeptide. Typically, at least 6, 8, 10, or 12 contiguous amino acids are required to form an epitope. However, epitopes which involve non-contiguous amino acids may require more, *e.g.*, at least 15, 25, or 50 amino acids.

An antibody which specifically binds to an epitope of a human α_{1a} adrenergic receptor-like GPCR polypeptide can be used therapeutically, as well as in immunochemical assays, such as Western blots, ELISAs, radioimmunoassays, immunohistochemical assays, immunoprecipitations, or other immunochemical assays known in the art. Various immunoassays can be used to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays are well known in the art. Such immunoassays typically involve the measurement of complex formation between an immunogen and an antibody which specifically binds to the immunogen.

Typically, an antibody which specifically binds to a human α_{1a} adrenergic receptor-like GPCR polypeptide provides a detection signal at least 5-, 10-, or 20-fold higher than a detection signal provided with other proteins when used in an immunochemical assay. Preferably, antibodies which specifically bind to human α_{1a} adrenergic receptor-like GPCR polypeptides do not detect other proteins in immunochemical assays and can immunoprecipitate a human α_{1a} adrenergic receptor-like GPCR polypeptide from solution.

Human α_{1a} adrenergic receptor-like GPCR polypeptides can be used to immunize a mammal, such as a mouse, rat, rabbit, guinea pig, monkey, or human, to produce polyclonal antibodies. If desired, a human α_{1a} adrenergic receptor-like GPCR polypeptide can be conjugated to a carrier protein, such as bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin. Depending on the host species, various adjuvants can be used to increase the immunological response. Such adjuvants include, but are not limited to, Freund's adjuvant, mineral gels (*e.g.*, aluminum hydroxide), and surface active substances (*e.g.* lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol). Among adjuvants used in humans, BCG (*bacilli Calmette-Guerin*) and *Corynebacterium parvum* are especially useful.

Monoclonal antibodies which specifically bind to a human α_{1a} adrenergic receptor-like GPCR polypeptide can be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These techniques include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique (Kohler *et al.*, *Nature 256*, 495-497, 1985; Kozbor *et al.*, *J. Immunol. Methods 81*, 31-42, 1985; Cote *et al.*, *Proc. Natl. Acad. Sci. 80*, 2026-2030, 1983; Cole *et al.*, *Mol. Cell Biol. 62*, 109-120, 1984).

In addition, techniques developed for the production of "chimeric antibodies," the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used (Morrison *et al.*, *Proc. Natl. Acad. Sci. 81*, 6851-6855, 1984; Neuberger *et al.*, *Nature 312*, 604-608, 1984; Takeda *et al.*, *Nature 314*, 452-454, 1985). Monoclonal and other antibodies also can be "humanized" to prevent a patient from mounting an immune response against the antibody when it is used therapeutically. Such antibodies may be sufficiently similar in sequence to human antibodies to be used directly in therapy or may require alteration of a few key residues. Sequence differences between rodent antibodies and human sequences can be minimized by replacing residues which differ from those in the human sequences by site directed mutagenesis of individual residues or by grating of entire complementarity determining regions. Alternatively, humanized antibodies can be produced using recombinant methods, as described in GB2188638B. Antibodies which specifically bind to a human α_{1a} adrenergic receptor-like GPCR polypeptide can contain antigen binding sites which are either partially or fully humanized, as disclosed in U.S. 5,565,332.

Alternatively, techniques described for the production of single chain antibodies can be adapted using methods known in the art to produce single chain antibodies which specifically bind to human α_{1a} adrenergic receptor-like GPCR polypeptides. Antibodies with related specificity, but of distinct idiotypic composition, can be

generated by chain shuffling from random combinatorial immunoglobin libraries (Burton, *Proc. Natl. Acad. Sci. 88*, 11120-23, 1991).

Single-chain antibodies also can be constructed using a DNA amplification method, such as PCR, using hybridoma cDNA as a template (Thirion *et al.*, 1996, *Eur. J. Cancer Prev. 5*, 507-11). Single-chain antibodies can be mono- or bispecific, and can be bivalent or tetravalent. Construction of tetravalent, bispecific single-chain antibodies is taught, for example, in Coloma & Morrison, 1997, *Nat. Biotechnol. 15*, 159-63. Construction of bivalent, bispecific single-chain antibodies is taught in Mallender & Voss, 1994, *J. Biol. Chem. 269*, 199-206.

A nucleotide sequence encoding a single-chain antibody can be constructed using manual or automated nucleotide synthesis, cloned into an expression construct using standard recombinant DNA methods, and introduced into a cell to express the coding sequence, as described below. Alternatively, single-chain antibodies can be produced directly using, for example, filamentous phage technology (Verhaar *et al.*, 1995, *Int. J. Cancer 61*, 497-501; Nicholls *et al.*, 1993, *J. Immunol. Meth. 165*, 81-91).

Antibodies which specifically bind to human α_{1a} adrenergic receptor-like GPCR polypeptides also can be produced by inducing *in vivo* production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature (Orlandi *et al.*, *Proc. Natl. Acad. Sci. 86*, 3833-3837, 1989; Winter *et al.*, *Nature 349*, 293-299, 1991).

Other types of antibodies can be constructed and used therapeutically in methods of the invention. For example, chimeric antibodies can be constructed as disclosed in WO 93/03151. Binding proteins which are derived from immunoglobulins and which are multivalent and multispecific, such as the "diabodies" described in WO 94/13804, also can be prepared.

Antibodies according to the invention can be purified by methods well known in the art. For example, antibodies can be affinity purified by passage over a column to which a human α_{1a} adrenergic receptor-like GPCR polypeptide is bound. The bound antibodies can then be eluted from the column using a buffer with a high salt concentration.

Antisense Oligonucleotides

Antisense oligonucleotides are nucleotide sequences which are complementary to a specific DNA or RNA sequence. Once introduced into a cell, the complementary nucleotides combine with natural sequences produced by the cell to form complexes and block either transcription or translation. Preferably, an antisense oligonucleotide is at least 11 nucleotides in length, but can be at least 12, 15, 20, 25, 30, 35, 40, 45, or 50 or more nucleotides long. Longer sequences also can be used. Antisense oligonucleotide molecules can be provided in a DNA construct and introduced into a cell as described above to decrease the level of human α_{1a} adrenergic receptor-like GPCR gene products in the cell.

Antisense oligonucleotides can be deoxyribonucleotides, ribonucleotides, or a combination of both. Oligonucleotides can be synthesized manually or by an automated synthesizer, by covalently linking the 5' end of one nucleotide with the 3' end of another nucleotide with non-phosphodiester internucleotide linkages such alkylphosphonates, phosphorothioates, phosphorodithioates, alkylphosphonothioates, alkylphosphonates, phosphoramidates, phosphate esters, carbamates, acetamidate, carboxymethyl esters, carbonates, and phosphate triesters. *See* Brown, *Meth. Mol. Biol. 20*, 1-8, 1994; Sonveaux, *Meth. Mol. Biol. 26*, 1-72, 1994; Uhlmann *et al.*, *Chem. Rev. 90*, 543-583, 1990.

Modifications of human α_{1a} adrenergic receptor-like GPCR gene expression can be obtained by designing antisense oligonucleotides which will form duplexes to the control, 5', or regulatory regions of the human α_{1a} adrenergic receptor-like GPCR

gene. Oligonucleotides derived from the transcription initiation site, *e.g.*, between positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or chaperons. Therapeutic advances using triplex DNA have been described in the literature (*e.g.*, Gee *et al.*, in Huber & Carr, MOLECULAR AND IMMUNOLOGIC APPROACHES, Futura Publishing Co., Mt. Kisco, N.Y., 1994). An antisense oligonucleotide also can be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Precise complementarity is not required for successful complex formation between an antisense oligonucleotide and the complementary sequence of a human α_{1a} adrenergic receptor-like GPCR polynucleotide. Antisense oligonucleotides which comprise, for example, 2, 3, 4, or 5 or more stretches of contiguous nucleotides which are precisely complementary to a human α_{1a} adrenergic receptor-like GPCR polynucleotide, each separated by a stretch of contiguous nucleotides which are not complementary to adjacent human α_{1a} adrenergic receptor-like GPCR nucleotides, can provide sufficient targeting specificity for human α_{1a} adrenergic receptor-like GPCR mRNA. Preferably, each stretch of complementary contiguous nucleotides is at least 4, 5, 6, 7, or 8 or more nucleotides in length. Non-complementary intervening sequences are preferably 1, 2, 3, or 4 nucleotides in length. One skilled in the art can easily use the calculated melting point of an antisense-sense pair to determine the degree of mismatching which will be tolerated between a particular antisense oligonucleotide and a particular human α_{1a} adrenergic receptor-like GPCR polynucleotide sequence.

Antisense oligonucleotides can be modified without affecting their ability to hybridize to a human α_{1a} adrenergic receptor-like GPCR polynucleotide. These modifications can be internal or at one or both ends of the antisense molecule. For example, internucleoside phosphate linkages can be modified by adding cholesteryl or diamine moieties with varying numbers of carbon residues between the amino

groups and terminal ribose. Modified bases and/or sugars, such as arabinose instead of ribose, or a 3', 5'-substituted oligonucleotide in which the 3' hydroxyl group or the 5' phosphate group are substituted, also can be employed in a modified antisense oligonucleotide. These modified oligonucleotides can be prepared by methods well known in the art. *See, e.g.*, Agrawal *et al.*, *Trends Biotechnol. 10*, 152-158, 1992; Uhlmann *et al.*, *Chem. Rev. 90*, 543-584, 1990; Uhlmann *et al.*, *Tetrahedron. Lett. 215*, 3539-3542, 1987.

Ribozymes

Ribozymes are RNA molecules with catalytic activity. *See, e.g.*, Cech, *Science 236*, 1532-1539; 1987; Cech, *Ann. Rev. Biochem. 59*, 543-568; 1990, Cech, *Curr. Opin. Struct. Biol. 2*, 605-609; 1992, Couture & Stinchcomb, *Trends Genet. 12*, 510-515, 1996. Ribozymes can be used to inhibit gene function by cleaving an RNA sequence, as is known in the art (*e.g.*, Haseloff *et al.*, U.S. Patent 5,641,673). The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Examples include engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of specific nucleotide sequences.

The coding sequence of a human α_{1a} adrenergic receptor-like GPCR polynucleotide can be used to generate ribozymes which will specifically bind to mRNA transcribed from the polynucleotide. Methods of designing and constructing ribozymes which can cleave other RNA molecules in trans in a highly sequence specific manner have been developed and described in the art (*see* Haseloff *et al. Nature 334*, 585-591, 1988). For example, the cleavage activity of ribozymes can be targeted to specific RNAs by engineering a discrete "hybridization" region into the ribozyme. The hybridization region contains a sequence complementary to the target RNA and thus specifically hybridizes with the target (see, for example, Gerlach *et al.*, EP 321,201).

Specific ribozyme cleavage sites within an RNA target can be identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target RNA containing the cleavage site can be evaluated for secondary structural features which may render the target inoperable. Suitability of candidate RNA targets also can be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays. Longer complementary sequences can be used to increase the affinity of the hybridization sequence for the target. The hybridizing and cleavage regions of the ribozyme can be integrally related such that upon hybridizing to the target RNA through the complementary regions, the catalytic region of the ribozyme can cleave the target.

Ribozymes can be introduced into cells as part of a DNA construct. Mechanical methods, such as microinjection, liposome-mediated transfection, electroporation, or calcium phosphate precipitation, can be used to introduce a ribozyme-containing DNA construct into cells in which it is desired to decrease human α_{1a} adrenergic receptor-like GPCR expression. Alternatively, if it is desired that the cells stably retain the DNA construct, the construct can be supplied on a plasmid and maintained as a separate element or integrated into the genome of the cells, as is known in the art. A ribozyme-encoding DNA construct can include transcriptional regulatory elements, such as a promoter element, an enhancer or UAS element, and a transcriptional terminator signal, for controlling transcription of ribozymes in the cells.

As taught in Haseloff *et al.*, U.S. Patent 5,641,673, ribozymes can be engineered so that ribozyme expression will occur in response to factors which induce expression of a target gene. Ribozymes also can be engineered to provide an additional level of regulation, so that destruction of mRNA occurs only when both a ribozyme and a target gene are induced in the cells.

Screening Methods

The invention provides assays for screening test compounds which bind to or modulate the activity of a human α_{1a} adrenergic receptor-like GPCR polypeptide or a polynucleotide. A test compound preferably binds to a human α_{1a} adrenergic receptor-like GPCR polypeptide or polynucleotide. More preferably, a test compound decreases or increases a biological activity mediated via human α_{1a} adrenergic receptor-like GPCR by at least about 10, preferably about 50, more preferably about 75, 90, or 100% relative to the absence of the test compound.

Test Compounds

Test compounds can be pharmacologic agents already known in the art or can be compounds previously unknown to have any pharmacological activity. The compounds can be naturally occurring or designed in the laboratory. They can be isolated from microorganisms, animals, or plants, and can be produced recombinantly, or synthesized by chemical methods known in the art. If desired, test compounds can be obtained using any of the numerous combinatorial library methods known in the art, including but not limited to, biological libraries, spatially addressable parallel solid phase or solution phase libraries, synthetic library methods requiring deconvolution, the "one-bead one-compound" library method, and synthetic library methods using affinity chromatography selection. The biological library approach is limited to polypeptide libraries, while the other four approaches are applicable to polypeptide, non-peptide oligomer, or small molecule libraries of compounds. *See* Lam, *Anticancer Drug Des. 12*, 145, 1997.

Methods for the synthesis of molecular libraries are well known in the art (*see*, for example, DeWitt *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 90*, 6909, 1993; Erb *et al. Proc. Natl. Acad. Sci. U.S.A. 91*, 11422, 1994; Zuckermann *et al.*, *J. Med. Chem. 37*, 2678, 1994; Cho *et al.*, *Science 261*, 1303, 1993; Carell *et al.*, *Angew. Chem. Int. Ed. Engl. 33*, 2059, 1994; Carell *et al.*, *Angew. Chem. Int. Ed. Engl. 33*, 2061; Gallop *et al.*, *J.*

Med. Chem. 37, 1233, 1994). Libraries of compounds can be presented in solution (*see, e.g.*, Houghten, *BioTechniques 13*, 412-421, 1992), or on beads (Lam, *Nature 354*, 82-84, 1991), chips (Fodor, *Nature 364*, 555-556, 1993), bacteria or spores (Ladner, U.S. Patent 5,223,409), plasmids (Cull *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 89*, 1865-1869, 1992), or phage (Scott & Smith, *Science 249*, 386-390, 1990; Devlin, *Science 249*, 404-406, 1990); Cwirla *et al.*, *Proc. Natl. Acad. Sci. 97*, 6378-6382, 1990; Felici, *J. Mol. Biol. 222*, 301-310, 1991; and Ladner, U.S. Patent 5,223,409).

High Throughput Screening

Test compounds can be screened for the ability to bind to human α_{1a} adrenergic receptor-like GPCR polypeptides or polynucleotides or to affect human α_{1a} adrenergic receptor-like GPCR activity or gene expression using high throughput screening. Using high throughput screening, many discrete compounds can be tested in parallel so that large numbers of test compounds can be quickly screened. The most widely established techniques utilize 96-well microtiter plates. The wells of the microtiter plates typically require assay volumes that range from 50 to 500 µl. In addition to the plates, many instruments, materials, pipettors, robotics, plate washers, and plate readers are commercially available to fit the 96-well format.

Alternatively, "free format assays," or assays that have no physical barrier between samples, can be used. For example, an assay using pigment cells (melanocytes) in a simple homogeneous assay for combinatorial peptide libraries is described by Jayawickreme *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 19,* 1614-18 (1994). The cells are placed under agarose in petri dishes, then beads that carry combinatorial compounds are placed on the surface of the agarose. The combinatorial compounds are partially released the compounds from the beads. Active compounds can be visualized as dark pigment areas because, as the compounds diffuse locally into the gel matrix, the active compounds cause the cells to change colors.

Another example of a free format assay is described by Chelsky, "Strategies for Screening Combinatorial Libraries: Novel and Traditional Approaches," reported at the First Annual Conference of The Society for Biomolecular Screening in Philadelphia, Pa. (Nov. 7-10, 1995). Chelsky placed a simple homogenous enzyme assay for carbonic anhydrase inside an agarose gel such that the enzyme in the gel would cause a color change throughout the gel. Thereafter, beads carrying combinatorial compounds via a photolinker were placed inside the gel and the compounds were partially released by UV-light. Compounds that inhibited the enzyme were observed as local zones of inhibition having less color change.

Yet another example is described by Salmon *et al.*, *Molecular Diversity 2*, 57-63 (1996). In this example, combinatorial libraries were screened for compounds that had cytotoxic effects on cancer cells growing in agar.

Another high throughput screening method is described in Beutel *et al.*, U.S. Patent 5,976,813. In this method, test samples are placed in a porous matrix. One or more assay components are then placed within, on top of, or at the bottom of a matrix such as a gel, a plastic sheet, a filter, or other form of easily manipulated solid support. When samples are introduced to the porous matrix they diffuse sufficiently slowly, such that the assays can be performed without the test samples running together.

Binding Assays

For binding assays, the test compound is preferably a small molecule which binds to and occupies the ligand binding site of the human α_{1a} adrenergic receptor-like GPCR polypeptide, thereby making the ligand binding site inaccessible to substrate such that normal biological activity is prevented. Examples of such small molecules include, but are not limited to, small peptides or peptide-like molecules. Potential ligands which bind to a polypeptide of the invention include, but are not limited to, the natural ligands of known GPCRs and analogues or derivatives thereof. Natural ligands of GPCRs include adrenomedullin, amylin, calcitonin gene related protein

(CGRP), calcitonin, anandamide, serotonin, histamine, norepinephrine, adrenalin, noradrenalin, platelet activating factor, thrombin, C5a, bradykinin, and chemokines.

In binding assays, either the test compound or the human α_{1a} adrenergic receptor-like GPCR polypeptide can comprise a detectable label, such as a fluorescent, radioisotopic, chemiluminescent, or enzymatic label, such as horseradish peroxidase, alkaline phosphatase, or luciferase. Detection of a test compound which is bound to the human α_{1a} adrenergic receptor-like GPCR polypeptide can then be accomplished, for example, by direct counting of radioemmission, by scintillation counting, or by determining conversion of an appropriate substrate to a detectable product.

Alternatively, binding of a test compound to a human α_{1a} adrenergic receptor-like GPCR polypeptide can be determined without labeling either of the interactants. For example, a microphysiometer can be used to detect binding of a test compound with a human α_{1a} adrenergic receptor-like GPCR polypeptide. A microphysiometer (*e.g.*, Cytosensor™) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a test compound and a human α_{1a} adrenergic receptor-like GPCR polypeptide (McConnell *et al.*, *Science 257*, 1906-1912, 1992).

Determining the ability of a test compound to bind to a human α_{1a} adrenergic receptor-like GPCR polypeptide also can be accomplished using a technology such as real-time Bimolecular Interaction Analysis (BIA) (Sjolander & Urbaniczky, *Anal. Chem. 63*, 2338-2345, 1991, and Szabo *et al.*, *Curr. Opin. Struct. Biol. 5*, 699-705, 1995). BIA is a technology for studying biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore™). Changes in the optical phenomenon surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

In yet another aspect of the invention, a human α_{1a} adrenergic receptor-like GPCR polypeptide can be used as a "bait protein" in a two-hybrid assay or three-hybrid assay (see, *e.g.*, U.S. Patent 5,283,317; Zervos *et al.*, *Cell 72*, 223-232, 1993; Madura *et al.*, *J. Biol. Chem. 268*, 12046-12054, 1993; Bartel *et al.*, *BioTechniques 14*, 920-924, 1993; Iwabuchi *et al.*, *Oncogene 8*, 1693-1696, 1993; and Brent W094/10300), to identify other proteins which bind to or interact with the human α_{1a} adrenergic receptor-like GPCR polypeptide and modulate its activity.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. For example, in one construct, polynucleotide encoding a human α_{1a} adrenergic receptor-like GPCR polypeptide can be fused to a polynucleotide encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct a DNA sequence that encodes an unidentified protein ("prey" or "sample") can be fused to a polynucleotide that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact *in vivo* to form an protein-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ), which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected, and cell colonies containing the functional transcription factor can be isolated and used to obtain the DNA sequence encoding the protein which interacts with the human α_{1a} adrenergic receptor-like GPCR polypeptide.

It may be desirable to immobilize either the human α_{1a} adrenergic receptor-like GPCR polypeptide (or polynucleotide) or the test compound to facilitate separation of bound from unbound forms of one or both of the interactants, as well as to accommodate automation of the assay. Thus, either the human α_{1a} adrenergic receptor-like GPCR polypeptide (or polynucleotide) or the test compound can be bound to a solid support. Suitable solid supports include, but are not limited to, glass

or plastic slides, tissue culture plates, microtiter wells, tubes, silicon chips, or particles such as beads (including, but not limited to, latex, polystyrene, or glass beads). Any method known in the art can be used to attach the human α_{1a} adrenergic receptor-like GPCR polypeptide (or polynucleotide) or test compound to a solid support, including use of covalent and non-covalent linkages, passive absorption, or pairs of binding moieties attached respectively to the polypeptide (or polynucleotide) or test compound and the solid support. Test compounds are preferably bound to the solid support in an array, so that the location of individual test compounds can be tracked. Binding of a test compound to a human α_{1a} adrenergic receptor-like GPCR polypeptide (or polynucleotide) can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and microcentrifuge tubes.

In one embodiment, the human α_{1a} adrenergic receptor-like GPCR polypeptide is a fusion protein comprising a domain that allows the polypeptide to be bound to a solid support. For example, glutathione-S-transferase fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, Mo.) or glutathione derivatized microtiter plates, which are then combined with the test compound or the test compound and the non-adsorbed human α_{1a} adrenergic receptor-like GPCR polypeptide; the mixture is then incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components. Binding of the interactants can be determined either directly or indirectly, as described above. Alternatively, the complexes can be dissociated from the solid support before binding is determined.

Other techniques for immobilizing proteins or polynucleotides on a solid support also can be used in the screening assays of the invention. For example, either a human α_{1a} adrenergic receptor-like GPCR polypeptide (or polynucleotide) or a test compound can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated human α_{1a} adrenergic receptor-like GPCR polypeptides (or poly-

nucleotides) or test compounds can be prepared from biotin-NHS(N-hydroxy-succinimide) using techniques well known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, Ill.) and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies which specifically bind to a human α_{1a} adrenergic receptor-like GPCR polypeptide, polynucleotide, or a test compound, but which do not interfere with a desired binding site, such as the active site of the human α_{1a} adrenergic receptor-like GPCR polypeptide, can be derivatized to the wells of the plate. Unbound target or protein can be trapped in the wells by antibody conjugation.

Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies which specifically bind to the human α_{1a} adrenergic receptor-like GPCR polypeptide or test compound, enzyme-linked assays which rely on detecting an activity of the human α_{1a} adrenergic receptor-like GPCR polypeptide, and SDS gel electrophoresis under non- reducing conditions.

Screening for test compounds which bind to a human α_{1a} adrenergic receptor-like GPCR polypeptide or polynucleotide also can be carried out in an intact cell. Any cell which comprises a human α_{1a} adrenergic receptor-like GPCR polypeptide or polynucleotide can be used in a cell-based assay system. A human α_{1a} adrenergic receptor-like GPCR polynucleotide can be naturally occurring in the cell or can be introduced using techniques such as those described above. Binding of the test compound to a human α_{1a} adrenergic receptor-like GPCR polypeptide or poly-nucleotide is determined as described above.

The specificity of binding of compounds showing affinity for the human α_{1a} adrenergic receptor-like GPCR is shown by comparing affinity to membranes obtained from transfected cell lines that express the receptor and membranes from cell lines or tissues known to express other types of α *(e.g.*, α_{1d}, α_{1b}) or beta adrenergic receptors. The specificity of binding of compounds showing affinity for

the human α_{1a} adrenergic receptor-like GPCR can be compared against the binding affinities to other types of alpha or beta adrenergic receptors. For example, the human alpha adrenergic receptor of the 1a subtype has been identified, cloned, and expressed (W094/08040 and WO 94/21660). Expression of cloned human α_{1d}, α_{1b} receptors and human α_{1a} adrenergic receptor-like GPCR and comparison of their binding properties with known selective antagonists provides a rational way for selection of compounds and discovery of new compounds with predictable pharmacological activities. Antagonism by these compounds of the human α_{1a} adrenergic receptor-like GPCR subtype may be functionally demonstrated in anesthetized animals. These compounds may be used to increase urine flow without exhibiting orthostatic hypotensive effects.

Compounds identified using the screening methods described above may further be defined by counterscreening. This is accomplished according to methods known in the art using other receptors responsible for mediating diverse biological functions. See, *e.g.*, W094/10989 and U.S. Patent 5,403,847. Compounds which are both selective amongst the various human α1 adrenergic receptor subtypes and which have low affinity for other receptors, such as the α2 adrenergic receptors, the β-adrenergic receptors, the muscarinic receptors, the serotonin receptors, and others are particularly preferred. The absence of these non-specific activities may be confirmed by using cloned and expressed receptors in an analogous fashion to the method disclosed herein for identifying compounds which have high affinity for the various human α1 adrenergic receptors. Furthermore, functional biological tests are used to confirm the effects of identified compounds as α1 a adrenergic receptor antagonists.

Functional Assays

Test compounds can be tested for the ability to increase or decrease a biological effect of a GPCR polypeptide. Such biological effects can be determined using the functional assays described in the specific examples, below. Functional assays can be carried out after contacting either a purified GPCR polypeptide, a cell membrane

preparation, or an intact cell with a test compound. A test compound which decreases a functional activity of a GPCR by at least about 10, preferably about 50, more preferably about 75, 90, or 100% is identified as a potential agent for decreasing GPCR activity. A test compound which increases GPCR activity by at least about 10, preferably about 50, more preferably about 75, 90, or 100% is identified as a potential agent for increasing GPCR activity.

One such screening procedure involves the use of melanophores which are transfected to express a GPCR polypeptide. Such a screening technique is described in WO 92/01810 published Feb. 6, 1992. Thus, for example, such an assay may be employed for screening for a compound which inhibits activation of the receptor polypeptide by contacting the melanophore cells which comprise the receptor with both the receptor ligand and a test compound to be screened. Inhibition of the signal generated by the ligand indicates that a test compound is a potential antagonist for the receptor, *i.e.*, inhibits activation of the receptor. The screen may be employed for identifying a test compound which activates the receptor by contacting such cells with compounds to be screened and determining whether each test compound generates a signal, *i.e.*, activates the receptor.

Other screening techniques include the use of cells which express a human GPCR polypeptide (for example, transfected CHO cells) in a system which measures extracellular pH changes caused by receptor activation (*see, e.g., Science 246*, 181-296, 1989). For example, test compounds may be contacted with a cell which expresses a human GPCR polypeptide and a second messenger response, *e.g.*, signal transduction or pH changes, can be measured to determine whether the test compound activates or inhibits the receptor.

Another such screening technique involves introducing RNA encoding a human GPCR polypeptide into *Xenopus* oocytes to transiently express the receptor. The transfected oocytes can then be contacted with the receptor ligand and a test compound to be screened, followed by detection of inhibition or activation of a

calcium signal in the case of screening for test compounds which are thought to inhibit activation of the receptor.

Another screening technique involves expressing a human GPCR polypeptide in cells in which the receptor is linked to a phospholipase C or D. Such cells include endothelial cells, smooth muscle cells, embryonic kidney cells, etc. The screening may be accomplished as described above by quantifying the degree of activation of the receptor from changes in the phospholipase activity.

Details of functional assays such as those described above are provided in the specific examples, below.

GPCR Gene Expression

In another embodiment, test compounds which increase or decrease GPCR gene expression are identified. A GPCR polynucleotide is contacted with a test compound, and the expression of an RNA or polypeptide product of the GPCR polynucleotide is determined. The level of expression of appropriate mRNA or polypeptide in the presence of the test compound is compared to the level of expression of mRNA or polypeptide in the absence of the test compound. The test compound can then be identified as a modulator of expression based on this comparison. For example, when expression of mRNA or polypeptide is greater in the presence of the test compound than in its absence, the test compound is identified as a stimulator or enhancer of the mRNA or polypeptide expression. Alternatively, when expression of the mRNA or polypeptide is less in the presence of the test compound than in its absence, the test compound is identified as an inhibitor of the mRNA or polypeptide expression.

The level of GPCR mRNA or polypeptide expression in the cells can be determined by methods well known in the art for detecting mRNA or polypeptide. Either qualitative or quantitative methods can be used. The presence of polypeptide

products of a GPCR polynucleotide can be determined, for example, using a variety of techniques known in the art, including immunochemical methods such as radioimmunoassay, Western blotting, and immunohistochemistry. Alternatively, polypeptide synthesis can be determined *in vivo*, in a cell culture, or in an *in vitro* translation system by detecting incorporation of labeled amino acids into a GPCR polypeptide.

Such screening can be carried out either in a cell-free assay system or in an intact cell. Any cell which expresses a GPCR polynucleotide can be used in a cell-based assay system. The GPCR polynucleotide can be naturally occurring in the cell or can be introduced using techniques such as those described above. Either a primary culture or an established cell line, such as CHO or human embryonic kidney 293 cells, can be used.

Pharmaceutical Compositions

The invention also provides pharmaceutical compositions which can be administered to a patient to achieve a therapeutic effect. Pharmaceutical compositions of the invention can comprise, for example, a GPCR polypeptide, GPCR polynucleotide, antibodies which specifically bind to a GPCR polypeptide, or mimetics, agonists, antagonists, or inhibitors of a GPCR polypeptide activity. The compositions can be administered alone or in combination with at least one other agent, such as stabilizing compound, which can be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions can be administered to a patient alone, or in combination with other agents, drugs or hormones.

In addition to the active ingredients, these pharmaceutical compositions can contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Pharmaceutical compositions of the invention can be

administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, parenteral, topical, sublingual, or rectal means. Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combination of active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents can be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

Dragee cores can be used in conjunction with suitable coatings, such as concentrated sugar solutions, which also can contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments can be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, *i.e.*, dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders, such as lactose or starches, lubricants, such as talc or magnesium

stearate, and, optionally, stabilizers. In soft capsules, the active compounds can be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration can be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions can contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds can be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Non-lipid polycationic amino polymers also can be used for delivery. Optionally, the suspension also can contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention can be manufactured in a manner that is known in the art, *e.g.*, by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes. The pharmaceutical composition can be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation can be a lyophilized powder which can contain any or all of the following: 1-50 mM histidine, 0.1%-2% sucrose, and 2-7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

Further details on techniques for formulation and administration can be found in the latest edition of REMINGTON'S PHARMACEUTICAL SCIENCES (Maack Publishing Co., Easton, Pa.). After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. Such labeling would include amount, frequency, and method of administration.

Therapeutic Indications and Methods

GPCRs are ubiquitous in the mammalian host and are responsible for many biological functions, including many pathologies. Accordingly, it is desirable to find compounds and drugs which stimulate a GPCR on the one hand and which can inhibit the function of a GPCR on the other hand. For example, compounds which activate a GPCR may be employed for therapeutic purposes, such as the treatment of asthma, Parkinson's disease, acute heart failure, urinary retention, and osteoporosis. In particular, compounds which activate GPCRs are useful in treating various cardiovascular ailments such as caused by the lack of pulmonary blood flow or hypertension. In addition these compounds may also be used in treating various physiological disorders relating to abnormal control of fluid and electrolyte homeostasis and in diseases associated with abnormal angiotensin-induced aldosterone secretion.

In general, compounds which inhibit activation of a GPCR can be used for a variety of therapeutic purposes, for example, for the treatment of hypotension and/or hypertension, angina pectoris, myocardial infarction, ulcers, asthma, allergies, benign prostatic hypertrophy, and psychotic and neurological disorders including schizophrenia, manic excitement, depression, delirium, dementia or severe mental retardation, dyskinesias, such as Huntington's disease or Tourett's syndrome, among others. Compounds which inhibit GPCRs also are useful in reversing endogenous anorexia, in the control of bulimia, and in treating various cardiovascular ailments such as caused by excessive pulmonary blood flow or hypotension.

Obesity and overweight are defined as an excess of body fat relative to lean body mass. An increase in caloric intake or a decrease in energy expenditure or both can bring about this imbalance leading to surplus energy being stored as fat. Obesity is associated with important medical morbidities and an increase in mortality. The causes of obesity are poorly understood and may be due to genetic factors, environmental factors or a combination of the two to cause a positive energy balance. In contrast, anorexia and cachexia are characterized by an imbalance in energy intake versus energy expenditure leading to a negative energy balance and weight loss. Agents that either increase energy expenditure and/or decrease energy intake, absorption or storage would be useful for treating obesity, overweight, and associated comorbidities. Agents that either increase energy intake and/or decrease energy expenditure or increase the amount of lean tissue would be useful for treating cachexia, anorexia and wasting disorders.

This gene, translated proteins and agents which modulate this gene or portions of the gene or its products are useful for treating obesity, overweight, anorexia, cachexia, wasting disorders, appetite suppression, appetite enhancement, increases or decreases in satiety, modulation of body weight, and/or other eating disorders such as bulimia. Also this gene, translated proteins and agents which modulate this gene or portions of the gene or its products are useful for treating obesity/overweight-associated comorbidities including hypertension; type 2 diabetes; coronary artery disease; hyperlipidemia; stroke; gallbladder disease; gout; osteoarthritis; sleep apnea and respiratory problems; some types of cancer including endometrial, breast, prostate and colon; thrombolic disease; polycystic ovarian syndrome; reduced fertility; complications of pregnancy; menstrual irregularities; hirsutism; stress incontinence and depression.

This invention further pertains to the use of novel agents identified by the screening assays described above. Accordingly, it is within the scope of this invention to use a test compound identified as described herein in an appropriate animal model. For

example, an agent identified as described herein (*e.g.*, a modulating agent, an antisense nucleic acid molecule, a specific antibody, ribozyme, or a human α_{1a} adrenergic receptor-like GPCR polypeptide binding molecule) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

Compounds identified using the screening methods above can be used, for example, for the treatment of BPH. Effects of blocking human α_{1a} adrenergic receptor-like GPCR include reduction of intra-ocular pressure, control of cardiac arrhythmias, and possibly a host of α_{1c} receptor mediated central nervous system events.

A reagent which affects human α_{1a} adrenergic receptor-like GPCR activity can be administered to a human cell, either *in vitro* or *in vivo*, to reduce human α_{1a} adrenergic receptor-like GPCR activity. The reagent preferably binds to an expression product of a human α_{1a} adrenergic receptor-like GPCR gene. If the expression product is a protein, the reagent is preferably an antibody. For treatment of human cells *ex vivo*, an antibody can be added to a preparation of stem cells which have been removed from the body. The cells can then be replaced in the same or another human body, with or without clonal propagation, as is known in the art.

In one embodiment, the reagent is delivered using a liposome. Preferably, the liposome is stable in the animal into which it has been administered for at least about 30 minutes, more preferably for at least about 1 hour, and even more preferably for at least about 24 hours. A liposome comprises a lipid composition that is capable of targeting a reagent, particularly a polynucleotide, to a particular site in an animal, such as a human. Preferably, the lipid composition of the liposome is capable of targeting to a specific organ of an animal, such as the lung, liver, spleen, heart brain, lymph nodes, and skin.

A liposome useful in the present invention comprises a lipid composition that is capable of fusing with the plasma membrane of the targeted cell to deliver its contents to the cell. Preferably, the transfection efficiency of a liposome is about 0.5 μg of DNA per 16 nmole of liposome delivered to about 10^6 cells, more preferably about 1.0 μg of DNA per 16 nmole of liposome delivered to about 10^6 cells, and even more preferably about 2.0 μg of DNA per 16 nmol of liposome delivered to about 10^6 cells. Preferably, a liposome is between about 100 and 500 nm, more preferably between about 150 and 450 nm, and even more preferably between about 200 and 400 nm in diameter.

Suitable liposomes for use in the present invention include those liposomes standardly used in, for example, gene delivery methods known to those of skill in the art. More preferred liposomes include liposomes having a polycationic lipid composition and/or liposomes having a cholesterol backbone conjugated to polyethylene glycol. Optionally, a liposome comprises a compound capable of targeting the liposome to a tumor cell, such as a tumor cell ligand exposed on the outer surface of the liposome.

Complexing a liposome with a reagent such as an antisense oligonucleotide or ribozyme can be achieved using methods which are standard in the art (see, for example, U.S. Patent 5,705,151). Preferably, from about 0.1 μg to about 10 μg of polynucleotide is combined with about 8 nmol of liposomes, more preferably from about 0.5 μg to about 5 μg of polynucleotides are combined with about 8 nmol liposomes, and even more preferably about 1.0 μg of polynucleotides is combined with about 8 nmol liposomes.

In another embodiment, antibodies can be delivered to specific tissues *in vivo* using receptor-mediated targeted delivery. Receptor-mediated DNA delivery techniques are taught in, for example, Findeis *et al. Trends in Biotechnol. 11*, 202-05 (1993); Chiou *et al.*, GENE THERAPEUTICS: METHODS AND APPLICATIONS OF DIRECT GENE

TRANSFER (J.A. Wolff, ed.) (1994); Wu & Wu, *J. Biol. Chem. 263*, 621-24 (1988); Wu *et al.*, *J. Biol. Chem. 269*, 542-46 (1994); Zenke *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 87*, 3655-59 (1990); Wu *et al.*, *J. Biol. Chem. 266*, 338-42 (1991).

Determination of a Therapeutically Effective Dose

The determination of a therapeutically effective dose is well within the capability of those skilled in the art. A therapeutically effective dose refers to that amount of active ingredient which increases or decreases human α_{1a} adrenergic receptor-like GPCR activity relative to the human α_{1a} adrenergic receptor-like GPCR activity which occurs in the absence of the therapeutically effective dose.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models, usually mice, rabbits, dogs, or pigs. The animal model also can be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

Therapeutic efficacy and toxicity, *e.g.*, ED_{50} (the dose therapeutically effective in 50% of the population) and LD_{50} (the dose lethal to 50% of the population), can be determined by standard pharmaceutical procedures in cell cultures or experimental animals. The dose ratio of toxic to therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD_{50}/ED_{50}.

Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active ingredient or to maintain the desired effect. Factors which can be taken into account include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions can be administered every 3 to 4 days, every week, or once every two weeks depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts can vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

If the reagent is a single-chain antibody, polynucleotides encoding the antibody can be constructed and introduced into a cell either *ex vivo* or *in vivo* using well-established techniques including, but not limited to, transferrin-polycation-mediated DNA transfer, transfection with naked or encapsulated nucleic acids, liposome-mediated cellular fusion, intracellular transportation of DNA-coated latex beads, protoplast fusion, viral infection, electroporation, "gene gun," and DEAE- or calcium phosphate-mediated transfection.

Effective *in vivo* dosages of an antibody are in the range of about 5 μg to about 50 μg/kg, about 50 μg to about 5 mg/kg, about 100 μg to about 500 μg/kg of patient body weight, and about 200 to about 250 μg/kg of patient body weight. For administration of polynucleotides encoding single-chain antibodies, effective *in vivo* dosages are in the range of about 100 ng to about 200 ng, 500 ng to about 50 mg,

about 1 μg to about 2 mg, about 5 μg to about 500 μg, and about 20 μg to about 100 μg of DNA.

If the expression product is mRNA, the reagent is preferably an antisense oligonucleotide or a ribozyme. Polynucleotides which express antisense oligonucleotides or ribozymes can be introduced into cells by a variety of methods, as described above.

Preferably, a reagent reduces expression of a human α_{1a} adrenergic receptor-like GPCR gene or the activity of a human α_{1a} adrenergic receptor-like GPCR polypeptide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% relative to the absence of the reagent. The effectiveness of the mechanism chosen to decrease the level of expression of a human α_{1a} adrenergic receptor-like GPCR gene or the activity of a human α_{1a} adrenergic receptor-like GPCR polypeptide can be assessed using methods well known in the art, such as hybridization of nucleotide probes to human α_{1a} adrenergic receptor-like GPCR-specific mRNA, quantitative RT-PCR, immunologic detection of a human α_{1a} adrenergic receptor-like GPCR polypeptide, or measurement of human α_{1a} adrenergic receptor-like GPCR activity.

In any of the embodiments described above, any of the pharmaceutical compositions of the invention can be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy can be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents can act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

Any of the therapeutic methods described above can be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

Diagnostic Methods

GPCRs also can be used in diagnostic assays for detecting diseases and abnormalities or susceptibility to diseases and abnormalities related to the presence of mutations in the nucleic acid sequences which encode a GPCR. Such diseases, by way of example, are related to cell transformation, such as tumors and cancers, and various cardiovascular disorders, including hypertension and hypotension, as well as diseases arising from abnormal blood flow, abnormal angiotensin-induced aldosterone secretion, and other abnormal control of fluid and electrolyte homeostasis.

Differences can be determined between the cDNA or genomic sequence encoding a GPCR in individuals afflicted with a disease and in normal individuals. If a mutation is observed in some or all of the afflicted individuals but not in normal individuals, then the mutation is likely to be the causative agent of the disease.

Sequence differences between a reference gene and a gene having mutations can be revealed by the direct DNA sequencing method. In addition, cloned DNA segments can be employed as probes to detect specific DNA segments. The sensitivity of this method is greatly enhanced when combined with PCR. For example, a sequencing primer can be used with a double-stranded PCR product or a single-stranded template molecule generated by a modified PCR. The sequence determination is performed by conventional procedures using radiolabeled nucleotides or by automatic sequencing procedures using fluorescent tags.

Genetic testing based on DNA sequence differences can be carried out by detection of alteration in electrophoretic mobility of DNA fragments in gels with or without denaturing agents. Small sequence deletions and insertions can be visualized, for

example, by high resolution gel electrophoresis. DNA fragments of different sequences can be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperatures (*see, e.g.*, Myers *et al.*, *Science 230*, 1242, 1985). Sequence changes at specific locations can also be revealed by nuclease protection assays, such as RNase and S 1 protection or the chemical cleavage method (*e.g.*, Cotton *et al.*, *Proc. Natl. Acad. Sci. USA 85*, 4397-4401, 1985). Thus, the detection of a specific DNA sequence can be performed by methods such as hybridization, RNase protection, chemical cleavage, direct DNA sequencing or the use of restriction enzymes and Southern blotting of genomic DNA. In addition to direct methods such as gel-electrophoresis and DNA sequencing, mutations can also be detected by *in situ* analysis.

Altered levels of a GPCR also can be detected in various tissues. Assays used to detect levels of the receptor polypeptides in a body sample, such as blood or a tissue biopsy, derived from a host are well known to those of skill in the art and include radioimmunoassays, competitive binding assays, Western blot analysis, and ELISA assays.

All patents and patent applications cited in this disclosure are expressly incorporated herein by reference. The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples which are provided for purposes of illustration only and are not intended to limit the scope of the invention.

EXAMPLE 1

Detection of α_{1a} adrenergic receptor-like GPCR activity

The polynucleotide of SEQ ID NO. 2 is inserted into the expression vector pCEV4 and the expression vector pCEV4-α_{1a} adrenergic receptor-like GPCR polypeptide obtained is transfected into human embryonic kidney 293 cells. The cells are scraped from a culture flask into 5 ml of Tris HCl, 5 mM EDTA, pH 7.5, and lysed by sonication. Cell lysates are centrifuged at 1000 rpm for 5 minutes at 4°C. The supernatant is centrifuged at 30,000 x g for 20 minutes at 4°C. The pellet is suspended in binding buffer containing 50 mM Tris HCl, 5 mM $MgSO_4$, 1 mM EDTA, 100 mM NaCl, pH 7.5, supplemented with 0.1 % BSA, 2 μg/ml aprotinin, 0.5 mg/ml leupeptin, and 10 μg/ml phosphoramidon. Optimal membrane suspension dilutions, defined as the protein concentration required to bind less than 10 % of an added radioligand, i.e. ^{125}I-labeled norepinephrine, are added to 96-well polypropylene microtiter plates containing ligand, non-labeled peptides, and binding buffer to a final volume of 250 μl.

In equilibrium saturation binding assays, membrane preparations are incubated in the presence of increasing concentrations (0.1 nM to 4 nM) of ^{125}I ligand.

Binding reaction mixtures are incubated for one hour at 30°C. The reaction is stopped by filtration through GF/B filters treated with 0.5% polyethyleneimine, using a cell harvester. Radioactivity is measured by scintillation counting, and data are analyzed by a computerized non-linear regression program. Non-specific binding is defined as the amount of radioactivity remaining after incubation of membrane protein in the presence of 100 nM of unlabeled peptide. Protein concentration is measured by the Bradford method using Bio-Rad Reagent, with bovine serum albumin as a standard. The α_{1a} adrenergic receptor-like GPCR activity of the polypeptide comprising the amino acid sequence of SEQ ID NO. 3 is demonstrated.

EXAMPLE 2

Radioligand binding assays

Human embryonic kidney 293 cells transfected with a polynucleotide which expresses human α_{1a} adrenergic receptor-like GPCR are scraped from a culture flask into 5 ml of Tris HCl, 5 mM EDTA, pH 7.5, and lysed by sonication. Cell lysates are centrifuged at 1000 rpm for 5 minutes at 4°C. The supernatant is centrifuged at 30,000 x g for 20 minutes at 4°C. The pellet is suspended in binding buffer containing 50 mM Tris HCl, 5 mM $MgSO_4$, 1 mM EDTA, 100 mM NaCl, pH 7.5, supplemented with 0.1 % BSA, 2 μg/ml aprotinin, 0.5 mg/ml leupeptin, and 10 μg/ml phosphoramidon. Optimal membrane suspension dilutions, defined as the protein concentration required to bind less than 10 % of the added radioligand, i.e. norepinephrine, are added to 96-well polypropylene microtiter plates containing ^{125}I-labeled ligand or test compound, non-labeled peptides, and binding buffer to a final volume of 250 μl.

In equilibrium saturation binding assays, membrane preparations are incubated in the presence of increasing concentrations (0.1 nM to 4 nM) of ^{125}I-labeled ligand or test compound (specific activity 2200 Ci/mmol). The binding affinities of different test compounds are determined in equilibrium competition binding assays, using 0.1 nM ^{125}I- peptide in the presence of twelve different concentrations of each test compound.

Binding reaction mixtures are incubated for one hour at 30°C. The reaction is stopped by filtration through GF/B filters treated with 0.5% polyethyleneimine, using a cell harvester. Radioactivity is measured by scintillation counting, and data are analyzed by a computerized non-linear regression program.

Non-specific binding is defined as the amount of radioactivity remaining after incubation of membrane protein in the presence of 100 nM of unlabeled peptide.

Protein concentration is measured by the Bradford method using Bio-Rad Reagent, with bovine serum albumin as a standard. A test compound which increases the radioactivity of membrane protein by at least 15% relative to radioactivity of membrane protein which was not incubated with a test compound is identified as a compound which binds to a human α_{1a} adrenergic receptor-like GPCR polypeptide.

EXAMPLE 3

Effect of a test compound on human α_{1a} adrenergic receptor-like GPCR-*mediated cyclic AMP formation*

Receptor-mediated inhibition of cAMP formation can be assayed in host cells which express human α_{1a} adrenergic receptor-like GPCR. Cells are plated in 96-well plates and incubated in Dulbecco's phosphate buffered saline (PBS) supplemented with 10 mM HEPES, 5 mM theophylline, 2 μg/ml aprotinin, 0.5 mg/ml leupeptin, and 10 μg/ml phosphoramidon for 20 minutes at 37°C in 5% CO2. A test compound is added and incubated for an additional 10 minutes at 37°C. The medium is aspirated, and the reaction is stopped by the addition of 100 mM HCl. The plates are stored at 4°C for 15 minutes. cAMP content in the stopping solution is measured by radioimmunoassay.

Radioactivity is quantified using a gamma counter equipped with data reduction software. A test compound which decreases radioactivity of the contents of a well relative to radioactivity of the contents of a well in the absence of the test compound is identified as a potential inhibitor of cAMP formation. A test compound which increases radioactivity of the contents of a well relative to radioactivity of the contents of a well in the absence of the test compound is identified as a potential enhancer of cAMP formation.

EXAMPLE 4

Effect of a test compound on the mobilization of intracellular calcium

Intracellular free calcium concentration can be measured by microspectrofluorometry using the fluorescent indicator dye Fura-2/AM (Bush *et al., J. Neurochem. 57*, 562-74, 1991). Stably transfected cells are seeded onto a 35 mm culture dish containing a glass coverslip insert. Cells are washed with HBS , incubated with a test compound, and loaded with 100 µl of Fura-2/AM (10 µM) for 20-40 minutes. After washing with HBS to remove the Fura-2/AM solution, cells are equilibrated in HBS for 10-20 minutes. Cells are then visualized under the 40X objective of a Leitz Fluovert FS microscope.

Fluorescence emission is determined at 510 nM, with excitation wavelengths alternating between 340 nM and 380 nM. Raw fluorescence data are converted to calcium concentrations using standard calcium concentration curves and software analysis techniques. A test compound which increases the fluorescence by at least 15% relative to fluorescence in the absence of a test compound is identified as a compound which mobilizes intracellular calcium.

EXAMPLE 5

Effect of a test compound on phosphoinositide metabolism

Cells which stably express human α_{1a} adrenergic receptor-like GPCR cDNA are plated in 96-well plates and grown to confluence. The day before the assay, the growth medium is changed to 100 µl of medium containing 1% serum and 0.5 µCi ^{3}H-myinositol. The plates are incubated overnight in a CO_2 incubator (5% CO_2 at 37°C). Immediately before the assay, the medium is removed and replaced by 200 µl of PBS containing 10 mM LiCl, and the cells are equilibrated with the new medium

for 20 minutes. During this interval, cells also are equilibrated with antagonist, added as a 10 µl aliquot of a 20-fold concentrated solution in PBS.

The ^{3}H-inositol phosphate accumulation from inositol phospholipid metabolism is started by adding 10 µl of a solution containing a test compound. To the first well 10 µl are added to measure basal accumulation. Eleven different concentrations of test compound are assayed in the following 11 wells of each plate row. All assays are performed in duplicate by repeating the same additions in two consecutive plate rows.

The plates are incubated in a CO_2 incubator for one hour. The reaction is terminated by adding 15 µl of 50% v/v trichloroacetic acid (TCA), followed by a 40 minute incubation at 4°C. After neutralizing TCA with 40 µl of 1 M Tris, the content of the wells is transferred to a Multiscreen HV filter plate (Millipore) containing Dowex AG1-X8 (200-400 mesh, formate form). The filter plates are prepared by adding 200 µl of Dowex AG1-X8 suspension (50% v/v, water:resin) to each well. The filter plates are placed on a vacuum manifold to wash or elute the resin bed. Each well is washed 2 times with 200 µl of water, followed by 2 x 200 µl of 5 mM sodium tetraborate/60 mM ammonium formate.

The ^{3}H-IPs are eluted into empty 96-well plates with 200 µl of 1.2 M ammonium formate/0.1 formic acid. The content of the wells is added to 3 ml of scintillation cocktail, and radioactivity is determined by liquid scintillation counting.

EXAMPLE 6

Receptor Binding Methods

Standard Binding Assays. Binding assays are carried out in a binding buffer containing 50 mM HEPES, pH 7.4, 0.5% BSA, and 5 mM $MgCl_2$. The standard assay for radioligand binding to membrane fragments comprising human α_{1a}

adrenergic receptor-like GPCR polypeptides is carried out as follows in 96 well microtiter plates (*e.g.*, Dynatech Immulon II Removawell plates). Radioligand is diluted in binding buffer+ PMSF/Baci to the desired cpm per 50 µl, then 50 µl aliquots are added to the wells. For non-specific binding samples, 5 µl of 40 µM cold ligand also is added per well. Binding is initiated by adding 150 µl per well of membrane diluted to the desired concentration (10-30 µg membrane protein/well) in binding buffer+ PMSF/Baci. Plates are then covered with Linbro mylar plate sealers (Flow Labs) and placed on a Dynatech Microshaker II. Binding is allowed to proceed at room temperature for 1-2 hours and is stopped by centrifuging the plate for 15 minutes at 2,000 x g. The supernatants are decanted, and the membrane pellets are washed once by addition of 200 µl of ice cold binding buffer, brief shaking, and recentrifugation. The individual wells are placed in 12 x 75 mm tubes and counted in an LKB Gammamaster counter (78% efficiency). Specific binding by this method is identical to that measured when free ligand is removed by rapid (3-5 seconds) filtration and washing on polyethyleneimine-coated glass fiber filters.

Three variations of the standard binding assay are also used.

1. Competitive radioligand binding assays with a concentration range of cold ligand vs. ^{125}I-labeled ligand are carried out as described above with one modification. All dilutions of ligands being assayed are made in 40X PMSF/Baci to a concentration 40X the final concentration in the assay. Samples of peptide (5 µl each) are then added per microtiter well. Membranes and radioligand are diluted in binding buffer without protease inhibitors. Radioligand is added and mixed with cold ligand, and then binding is initiated by addition of membranes.

2. Chemical cross-linking of radioligand with receptor is done after a binding step identical to the standard assay. However, the wash step is done with binding buffer minus BSA to reduce the possibility of non-specific cross-

linking of radioligand with BSA. The cross-linking step is carried out as described below.

3. Larger scale binding assays to obtain membrane pellets for studies on solubilization of receptor:ligand complex and for receptor purification are also carried out. These are identical to the standard assays except that (a) binding is carried out in polypropylene tubes in volumes from 1-250 ml, (b) concentration of membrane protein is always 0.5 mg/ml, and (c) for receptor purification, BSA concentration in the binding buffer is reduced to 0.25%, and the wash step is done with binding buffer without BSA, which reduces BSA contamination of the purified receptor.

EXAMPLE 7

Chemical Cross-Linking of Radioligand to Receptor

After a radioligand binding step as described above, membrane pellets are resuspended in 200 µl per microtiter plate well of ice-cold binding buffer without BSA. Then 5 µl per well of 4 mM N-5-azido-2-nitrobenzoyloxysuccinimide (ANB-NOS, Pierce) in DMSO is added and mixed. The samples are held on ice and UV-irradiated for 10 minutes with a Mineralight R-52G lamp (UVP Inc., San Gabriel, Calif.) at a distance of 5-10 cm. Then the samples are transferred to Eppendorf microfuge tubes, the membranes pelleted by centrifugation, supernatants removed, and membranes solubilized in Laemmli SDS sample buffer for polyacrylamide gel electrophoresis (PAGE). PAGE is carried out as described below. Radiolabeled proteins are visualized by autoradiography of the dried gels with Kodak XAR film and Dupont image intensifier screens.

EXAMPLE 8

Membrane Solubilization

Membrane solubilization is carried out in buffer containing 25 mM Tris , pH 8, 10% glycerol (w/v) and 0.2 mM $CaCl_2$ (solubilization buffer). The highly soluble detergents including Triton X-100, deoxycholate, deoxycholate:lysolecithin, CHAPS, and zwittergent are made up in solubilization buffer at 10% concentrations and stored as frozen aliquots. Lysolecithin is made up fresh because of insolubility upon freeze-thawing and digitonin is made fresh at lower concentrations due to its more limited solubility.

To solubilize membranes, washed pellets after the binding step are resuspended free of visible particles by pipetting and vortexing in solubilization buffer at 100,000 x g for 30 minutes. The supernatants are removed and held on ice and the pellets are discarded.

EXAMPLE 9

Assay of Solubilized Receptors

After binding of ^{125}I ligands and solubilization of the membranes with detergent, the intact R:L complex can be assayed by four different methods. All are carried out on ice or in a cold room at 4-10°C.).

1. Column chromatography (Knuhtsen *et al.*, *Biochem. J. 254*, 641-647, 1988). Sephadex G-50 columns (8 x 250 mm) are equilibrated with solubilization buffer containing detergent at the concentration used to solubilize membranes and 1 mg/ml bovine serum albumin. Samples of solubilized membranes (0.2-0.5 ml) are applied to the columns and eluted at a flow rate of about 0.7 ml/minute. Samples (0.18 ml) are collected. Radioactivity is determined

in a gamma counter. Void volumes of the columns are determined by the elution volume of blue dextran. Radioactivity eluting in the void volume is considered bound to protein. Radioactivity eluting later, at the same volume as free ^{125}I ligands, is considered non-bound.

2. Polyethyleneglycol precipitation (Cuatrecasas, *Proc. Natl. Acad. Sci. USA 69*, 318-322, 1972). For a 100 µl sample of solubilized membranes in a 12 x 75 mm polypropylene tube, 0.5 ml of 1% (w/v) bovine gamma globulin (Sigma) in 0.1 M sodium phosphate buffer is added, followed by 0.5 ml of 25% (w/v) polyethyleneglycol (Sigma) and mixing. The mixture is held on ice for 15 minutes. Then 3 ml of 0.1 M sodium phosphate, pH 7.4, is added per sample. The samples are rapidly (1-3 seconds) filtered over Whatman GF/B glass fiber filters and washed with 4 ml of the phosphate buffer. PEG-precipitated receptor : ^{125} I-ligand complex is determined by gamma counting of the filters.

3. GFB/PEI filter binding (Bruns *et al.*, *Analytical Biochem. 132*, 74-81, 1983). Whatman GF/B glass fiber filters are soaked in 0.3% polyethyleneimine (PEI, Sigma) for 3 hours. Samples of solubilized membranes (25-100 µl) are replaced in 12 x 75 mm polypropylene tubes. Then 4 ml of solubilization buffer without detergent is added per sample and the samples are immediately filtered through the GFB/PEI filters (1-3 seconds) and washed with 4 ml of solubilization buffer. CPM of receptor : ^{125} I-ligand complex adsorbed to filters are determined by gamma counting.

4. Charcoal/Dextran (Paul and Said, *Peptides 7[Suppl. 1]*,147-149, 1986). Dextran T70 (0.5 g, Pharmacia) is dissolved in 1 liter of water, then 5 g of activated charcoal (Norit A, alkaline; Fisher Scientific) is added. The suspension is stirred for 10 minutes at room temperature and then stored at 4°C. until use. To measure R:L complex, 4 parts by volume of charcoal/-dextran suspension are added to 1 part by volume of solubilized membrane.

The samples are mixed and held on ice for 2 minutes and then centrifuged for 2 minutes at 11,000 x g in a Beckman microfuge. Free radioligand is adsorbed charcoal/dextran and is discarded with the pellet. Receptor : ^{125} I-ligand complexes remain in the supernatant and are determined by gamma counting.

EXAMPLE 10

Receptor Purification

Binding of biotinyl-receptor to GH_4 C1 membranes is carried out as described above. Incubations are for 1 hour at room temperature. In the standard purification protocol, the binding incubations contain 10 nM Bio-S29. ^{125}I ligand is added as a tracer at levels of 5,000-100,000 cpm per mg of membrane protein. Control incubations contain 10 μM cold ligand to saturate the receptor with non-biotinylated ligand.

Solubilization of receptor:ligand complex also is carried out as described above, with 0.15% deoxycholate:lysolecithin in solubilization buffer containing 0.2 mM $MgCl_2$, to obtain 100,000 x g supernatants containing solubilized R:L complex.

Immobilized streptavidin (streptavidin cross-linked to 6% beaded agarose, Pierce Chemical Co.; "SA-agarose") is washed in solubilization buffer and added to the solubilized membranes as 1/30 of the final volume. This mixture is incubated with constant stirring by end-over-end rotation for 4-5 hours at 4-10 °C. Then the mixture is applied to a column and the non-bound material is washed through. Binding of radioligand to SA-agarose is determined by comparing cpm in the 100,000 x g supernatant with that in the column effluent after adsorption to SA-agarose. Finally, the column is washed with 12-15 column volumes of solubilization buffer+0.15% deoxycholate:lysolecithin +1/500 (vol/vol) 100 x 4pase.

The streptavidin column is eluted with solubilization buffer+0.1 mM EDTA+0.1 mM EGTA+0.1 mM GTP-gamma-S (Sigma)+0.15% (wt/vol) deoxycholate:lysolecithin +1/1000 (vol/vol) 100.times.4pase. First, one column volume of elution buffer is passed through the column and flow is stopped for 20-30 minutes. Then 3-4 more column volumes of elution buffer are passed through. All the eluates are pooled.

Eluates from the streptavidin column are incubated overnight (12-15 hours) with immobilized wheat germ agglutinin (WGA agarose, Vector Labs) to adsorb the receptor via interaction of covalently bound carbohydrate with the WGA lectin. The ratio (vol/vol) of WGA-agarose to streptavidin column eluate is generally 1:400. A range from 1:1000 to 1:200 also can be used. After the binding step, the resin is pelleted by centrifugation, the supernatant is removed and saved, and the resin is washed 3 times (about 2 minutes each) in buffer containing 50 mM HEPES, pH 8, 5 mM $MgCl_2$, and 0.15% deoxycholate:lysolecithin. To elute the WGA-bound receptor, the resin is extracted three times by repeated mixing (vortex mixer on low speed) over a 15-30 minute period on ice, with 3 resin columns each time, of 10 mM N-N'-N"-triacetylchitotriose in the same HEPES buffer used to wash the resin. After each elution step, the resin is centrifuged down and the supernatant is carefully removed, free of WGA-agarose pellets. The three, pooled eluates contain the final, purified receptor. The material non-bound to WGA contain G protein subunits specifically eluted from the streptavidin column, as well as non-specific contaminants. All these fractions are stored frozen at -90°C.

EXAMPLE 11

Identification of test compounds that bind to human α_{1a} adrenergic receptor-like GPCR polypeptides

Purified human α_{1a} adrenergic receptor-like GPCR polypeptides comprising a glutathione-S-transferase protein and absorbed onto glutathione-derivatized wells of 96-well microtiter plates are contacted with test compounds from a small molecule

library at pH 7.0 in a physiological buffer solution. Human α_{1a} adrenergic receptor-like GPCR polypeptides comprise an amino acid sequence shown in SEQ ID NO. 3. The test compounds comprise a fluorescent tag. The samples are incubated for 5 minutes to one hour. Control samples are incubated in the absence of a test compound.

The buffer solution containing the test compounds is washed from the wells. Binding of a test compound to a human α_{1a} adrenergic receptor-like GPCR polypeptide is detected by fluorescence measurements of the contents of the wells. A test compound which increases the fluorescence in a well by at least 15% relative to fluorescence of a well in which a test compound was not incubated is identified as a compound which binds to a human α_{1a} adrenergic receptor-like GPCR polypeptide.

EXAMPLE 12

Selective binding assays

Membranes prepared from stably transfected human α_{1d} and α_{1b} cell lines (ATCC CRL 11138 and CRL 11139, respectively) are used to identify compounds that selectively bind to the human α_{1a} adrenergic receptor-like GPCR. These competition binding reactions (total volume=200 µl) contain 50 mM Tris-HCl pH. 7.4, 5 mM EDTA, 150 mM NaCl, 100 pM ^{125} I-HEAT, membranes prepared from cell lines transfected with the respective α1 subtype expression plasmid, and increasing amounts of unlabeled ligand. Reactions are incubated at room temperature for one hour with shaking. Reactions are filtered onto Whatman GF/C glass fiber filters with an Inotec 96 well cell harvester. Filters are washed three times with ice cold buffer and bound radioactivity is determined (Ki).

EXAMPLE 13

Exemplary functional assays

In order to confirm the specificity of compounds for the human α_{1a} adrenergic receptor-like GPCR and to define the biological activity of the compounds, the following functional tests may be performed:

1. *In vitro* Rat, Dog and Human Prostate and Dog Urethra

Taconic Farms Sprague-Dawley male rats, weighing 250-400 grams are sacrificed by cervical dislocation under anesthesia (methohexital; 50 mg/kg, i.p.). An incision is made into the lower abdomen to remove the ventral lobes of the prostate. Each prostate removed from a mongrel dog is cut into 6-8 pieces longitudinally along the urethra opening and stored in ice-cold oxygenated Krebs solution overnight before use if necessary. Dog urethra proximal to prostate is cut into approximately 5 mm rings, the rings are then cut open for contractile measurement of circular muscles. Human prostate chips from transurethral surgery of benign prostate hyperplasia are also stored overnight in ice-cold Krebs solution if needed.

The tissue is placed in a Petri dish containing oxygenated Krebs solution (NaCl, 118 mM; KCl, 4.7 mM; $CaCl_2$, 2.5 mM; KH_2PO_4, 1.2 mM; $MgSO_4$, 1.2 mM; $NaHCO_3$, 2.0 mM; dextrose, 11 mM) warmed to 37 °C. Excess lipid material and connective tissue are carefully removed. Tissue segments are attached to glass tissue holders with 4-0 surgical silk and placed in a 5 ml jacketed tissue bath containing Krebs buffer at 37 °C., bubbled with 5% CO_2/95% O_2. The tissues are connected to a Statham-Gould force transducer; 1 gram (rat, human) or 1.5 gram (dog) of tension is applied and the tissues are allowed to equilibrate for one hour. Contractions are recorded on a Hewlett-Packard 7700 series strip chart recorder.

After a single priming dose of 3 μM (for rat), 10 μM (for dog) and 20 μM (for human) of phenylephrine, a cumulative concentration response curve to an agonist is generated; the tissues are washed every 10 minutes for one hour. Vehicle or antagonist is added to the bath and allowed to incubate for one hour, then another cumulative concentration response curve to the agonist is generated.

EC_{50} values are calculated for each group using GraphPad Inplot software. pA2 (approximately log Kb) values are obtained from Schild plot when three or more concentrations are tested.

2. Measurement of Intra-Urethral Pressure in Anesthetized Dogs

The following model is used to measure adrenergically mediated changes in intra-urethral pressure and arterial pressure in anesthetized dogs in order to evaluate the efficacy and potency of selective human α_{1a} adrenergic receptor-like GPCR antagonists. Male mongrel dogs (7-12 kg) are used in this study. The dogs are anesthetized with pentobarbital sodium (35 mg/kg, i.v. plus 4 mg/kg/hr iv infusion). An endotracheal tube is inserted and the animal ventilated with room air using a Harvard instruments positive displacement large animal ventilator. Catheters (PE 240 or 260) are placed in the aorta via the femoral artery and vena cava via the femoral veins (2 catheters, one in each vein) for the measurement of arterial pressure and the administration of drugs, respectively. A supra-pubic incision .about.1/2 inch lateral to the penis is made to expose the ureters, bladder and urethra. The urethers are ligated and cannulated so that urine flows freely into beakers. The dome of the bladder is retracted to facilitate dissection of the proximal and distal urethra. Umbilical tape is passed beneath the urethra at the bladder neck and another piece of umbilical tape is placed under the distal urethra approximately 1-2 cm distal to the prostate. The bladder is incised and a Millar micro-tip

pressure transducer is advanced into the urethra. The bladder incision is sutured with 2-0 or 3-0 silk (purse-string suture) to hold the transducer. The tip of the transducer is placed in the prostatic urethra and the position of the Millar catheter is verified by gently squeezing the prostate and noting the large change in urethral pressure.

Phenylephrine, an α1 adrenergic agonist, is administered (0.1-100 μg/kg, iv; 0.05 ml/kg volume) in order to construct dose response curves for changes in intra-urethral and arterial pressure. Following administration of increasing doses of an alpha adrenergic antagonist (or vehicle), the effects of phenylephrine on arterial pressure and intra-urethral pressure are re-evaluated. Four or five phenylephrine dose-response curves are generated in each animal (one control, three or four doses of antagonist or vehicle). The relative antagonist potency on phenylephrine induced changes in arterial and intra-urethral pressure are determined by Schild analysis. The family of averaged curves are fit simultaneously (using ALLFIT software package) with a four parameter logistic equation constraining the slope, minimum response, and maximum response to be constant among curves. The dose ratios for the antagonist doses (rightward shift in the dose-response curves from control) are calculated as the ratio of the ED_{50} s for the respective curves. These dose-ratios are then used to construct a Schild plot and the Kb (expressed as μg/kg, iv) determined. The Kb (dose of antagonist causing a 2-fold rightward shift of the phenylephrine dose-response curve) is used to compare the relative potency of the antagonists on inhibiting phenylephrine responses for intra-urethral and arterial pressure. The relative selectivity is calculated as the ratio of arterial pressure and intra-urethral pressure Kbs. Effects of the α1 antagonists on baseline arterial pressure are also monitored. Comparison of the relative antagonist potency on changes in arterial pressure and intra-urethral pressure provide insight as to whether the alpha receptor subtype responsible for increasing intra-urethral pressure is also present in the systemic vasculature. According to this method, one is able to confirm the

selectivity of α_{1a} adrenergic receptor antagonists that prevent the increase in intra-urethral pressure to phenylephrine without any activity at the vasculature.

EXAMPLE 14

Identification of a test compound which decreases human α_{1a} adrenergic receptor-like GPCR gene expression

A test compound is administered to a culture of human gastric cells and incubated at 37°C for 10 to 45 minutes. A culture of the same type of cells incubated for the same time without the test compound provides a negative control.

RNA is isolated from the two cultures as described in Chirgwin *et al.*, *Biochem. 18*, 5294-99, 1979). Northern blots are prepared using 20 to 30 µg total RNA and hybridized with a ^{32}P-labeled human α_{1a} adrenergic receptor-like GPCR-specific probe at 65°C in Express-hyb (CLONTECH). The probe comprises at least 11 contiguous nucleotides selected from the complement of SEQ ID NO.1. A test compound which decreases the human α_{1a} adrenergic receptor-like GPCR-specific signal relative to the signal obtained in the absence of the test compound is identified as an inhibitor of human α_{1a} adrenergic receptor-like GPCR gene expression.

EXAMPLE 15

Treatment of obesity with a reagent which specifically binds to a GPCR gene product

Synthesis of antisense human α_{1a} adrenergic receptor-like GPCR oligonucleotides comprising at least 11 contiguous nucleotides selected from the complement of SEQ ID NO. 2 is performed on a Pharmacia Gene Assembler series synthesizer using the phosphoramidite procedure (Uhlmann *et al.*, *Chem. Rev. 90*, 534-83, 1990).

Following assembly and deprotection, oligonucleotides are ethanol-precipitated twice, dried, and suspended in phosphate-buffered saline (PBS) at the desired concentration. Purity of these oligonucleotides is tested by capillary gel electrophoreses and ion exchange HPLC. Endotoxin levels in the oligonucleotide preparation are determined using the *Limulus* Amebocyte Assay (Bang, *Biol. Bull. (Woods Hole, Mass.) 105*, 361-362, 1953).

The antisense oligonucleotides are administered to a patient with obesity. The severity of the patient's obesity decreases.

EXAMPLE 16

Tissue specific expression

PCR analysis using cDNA phage libraries from human tissues

Human cDNA phage libraries (Stratagene) were purchased in a "human tissue panel IA" as described in Table1. One-half µl of each library-purchased sample were used as template in PCR analysis regardless the title (phage/ml) for non-quantitative expression analysis. In addition, a positive control PCR reaction was performed with about 20 ng of human genomic DNA as template, and a negative control was performed with no template.

The standard PCR procedures were as indicated by Perkin Elmer. The PCR protocol was as follows:

Primers:

Primer A: 5'-ACAAGGGTCGCACAGAGGTC-3'

Primer B: 5'-TGTTTCTCGTGTTATGGACAGTTCA-3'

PCR reaction mix:

0.5µl	template
1 x	Gold PCR Buffer (Perkin Elmer)
0.2 mM	dNTPs (Pharmacia)
1.5mM	$MgCl_2$ (Perkin Elmer)
0.5 µM	primer A
0.5 µM	primer B
2.5 U	AmpliTaq Gold DNA Polymerase (Perkin Elmer)

to 25 µl final reaction volume with sterile H_2O.

The amplification protocol was performed in Perkin Elmer 9700 thermocycler:

1 X the following step:

pre PCR	9' at 94°C

40 times the following steps:

denaturation	30'' at 94°C
annealing	1' at 56°C
elongation	30'' at 72°C

Expected length of specific PCR product: 560bp.

Amplification products were analyzed by electrophoresis on a 2% agarose (SeaKem LE agarose, FMC bioproducts) gel in 1XTAE running buffer following standard procedure, as described by Maniatis *et al.* PCR amplification products of the expected size were detectable in the positive control (human genomic DNA) and in lanes corresponding to the Brain (Corpus Striatum) cDNA phage library.

To check PCR product identity, a mixture of the amplification products obtained was used for restriction analysis with the enzyme ApaI (BioLabs) according to the manufacturer's instructions. Restriction fragments were analysed by electrophoresis

on 2% agarose (SeaKem LE agarose, FMC bioproducts) gel in 1 X TAE running buffer following standard procedure, as described by Maniatis *et al.* Restriction by ApaI produced two fragments of the expected size (about 240bp and 330bp).

PCR analysis using cDNAs from human tissues

Human QUICK-clone cDNAs (Clontech) were ordered in a "human tissue panel IB" (unless differently specified) as described in Table 2. One-half µl of each cDNA purchased sample were used as template in PCR for non quantitative expression analysis. In addition, a positive control PCR reaction was performed with about 20 ng of human genomic DNA as template, and a negative control was performed with no template.

The experimental protocol was as described above.

PCR amplification products of the expected size as shown in Fig. 9 were detectable in the positive control (human genomic DNA) and in lanes corresponding to the following cDNAs:

- Whole Brain
- Brain (hippocampus)
- Brain (cerebellum)
- Spinal cord – very faint amplification product
- Bladder
- Prostate
- Adrenal gland - very faint amplification product

Table 1

Library	Description	Catalog
Brain(corpus striatum)	Caudate and putamen, males, 57 & 63 years old	936213
Brain (fetal)	Male and female, Caucasian	937227
Brain (frontal cortex)	Female, 85 years old	936212
Brain (substantia nigra)	Male and female, 60 years old	936210
Brain (occipital cortex)	Female, 85 years old	936211
Brain stem	Female,2 years old	935206
Bronchial muscle	Human bronchial/tracheal smooth muscle primary cells	780032
Coronary	Coronary artery endothelial primary cells	780025
Coronary	Coronary artery smooth muscle primary cells	780029
Endothelial	Microvascular endothelial primary cells	780028
Heart	12 pooled, 19-50 years old, male/female Caucasian	937257
Kidney	8 pooled, whole kidney from 24-55 years old, male/female, Caucasian	937250
Liver	Normal,38 years old, Caucasian	937241
Lung	Male, 72 years old, normal	937210
Muscle (skeletal)	Female, 19 years old	936215
Ovary	Normal, 49 years old, Caucasian	937217
Pulmonary artery endothelial	Pulmonary artery endothelial primary cells	780027
Umbilical artery endothelial cells	Umbilical artery endothelial cells	780023
Whole Brain	Whole brain 60 year	HL5018t
Spinal chord	Whole, pooled from 26 male/female, 16-75 years	HL5001b

EXAMPLE 17

Cloning of full length cDNA

Full length cDNA was obtained by PCR using human genomic DNA as template. Standard PCR procedure was as indicated by Perkin Elmer.
PCR protocol was as follows:

Primers:

Primer C: 5'-TGCCTGCGTGTTTCTTTTGT-3'

Primer D: 5'-TCTTGTTTCTCGTGTTATGGACAGT-3'

PCR reaction mix:

0.5 µl	genomic DNA 50ng
5 µl	10x Stratagene Cloned Pfu Buffer,
0.2 mM	dNTPs (Pharmacia)
0.5 µM	primer C
0.5 µM	primer D
2.5 U	Pfu DNA Polymerase (Stratagene, Catalogue 600154)

to 50 µl final reaction volume with sterile H_2O.

The amplification protocol was performed in Perkin Elmer 9700 thermocycler:
1 time the following step:

pre PCR	4' at 94°C

30 times the following steps:

denaturation	1' at 94°C
annealing	1' at 56°C
elongation	1'30''at 72°C
1 time the following step:	7' at 72°C

The expected length of specific PCR product was 1694 bp.

PCR product length was controlled by electrophoresis on 1% agarose (SeaKem LE agarose, FMC bioproducts) gel in 1XTAE running buffer following standard procedure, as described by Maniatis *et al.* The electrophoretic band of the expected size was excised from the gel and purified by quiaquik gel extraction kit (Quiagen) following instructions.

A nested PCR reaction was done using the DNA obtained from the first PCR reaction as template.

Standard PCR procedure was as indicated by Perkin Elmer.

PCR protocol was as follows:

Primers:

Primer E: 5'-GGAATTCCGCCGCCATGGCGTCCACCTGCACCAACAGCAC-3'

Primer F: 5'-CGCGGATCCGCGTTCAAGGAAAAGTAGCAGAA-3'

PCR reaction mix:

0.5 µl	template
5 µl	10x Stratagene Cloned Pfu Buffer,
0.2 mM	dNTPs (Pharmacia)
0.5 µM	primer E
0.5 µM	primer F
2.5 U	Pfu DNA Polymerase (Stratagene, Catalogue 600154)

to 50 µl final reaction volume with sterile H_2O.

The amplification protocol was performed in Perkin Elmer 9700 thermocycler:

1 time the following step:

pre PCR	4' at 94°C

30 times the following steps:

denaturation	1' at 94°C
annealing	1' at 56°C
elongation	1'30'' at 72°C
1 time the following step:	10' at 72°C

After the PCR reaction, the PCR product was purified using a purification column (Quiaquick PCR Purification kit, catalogue 28104 Quiagen). After this step an "A-Tailing" reaction was performed by adding 0.2mM dATP (Pharmacia) and 2.5U AmpiTaq Gold DNA polymerase (Perkin Elmer) to the PCR product and incubating in a Perkin Elmer 9700 thermocycler:

10' at 72°C

The purified PCR product was cloned in pCR II TOPO™ vector (catalogue 45-0640, Invitrogen) with Rapid DNA Ligation Kit, (catalogue 1635-379, Roche Diagnostics) following standard procedure and manufacturer's instructions.

A recombinant clone, named #96pCRIITOPO, was selected and sequenced. Sequencing revealed that the recombinant plasmid contains the full length ORF as deposited in databank, with no mutations. See SEQ ID NOS. 7 and 8.

EXAMPLE 18

Subcloning of full length cDNA in a mammalian expression vector

The pIRES2-EGFP (Catalogue 6029-1, CLONTECH Laboratories, Inc.) vector was selected for expression in mammalian cells. This vector contains the internal

ribosome entry site of the encephalomyocarditis virus (ECMV) between the Multiple Cloning Sites and the enhanced green fluorescent protein (EGFP) coding region. This allows the gene of interest (cloned into the MCS) and the EGFP gene to be transcribed in a single bicistronic mRNA and therefore to use it for a highly efficient selection (by flow cytometry) of transfected cells.

The EcoRI-BamHI restriction fragment of human α_{1A} adrenergic receptor-like GPCR-pCRIITOPO was inserted in oRI-BamHI digested pIRES2-EGFP following standard procedure. Plasmid DNA was isolated from transformants and examined by restriction analysis for the presence of the correct fragment and correct 5'-3' orientation.

EXAMPLE 19

Quantitative analysis of relative expression of α_{1a} adrenergic receptor-like GPCR in human tissues

Quantitative expression profiling was performed by the form of quantitative PCR analysis called "kinetic analysis" firstly described in Higuchi *et al.*, 1992 and Higuchi et al., 1993. The principle is that at any given cycle within the exponential phase of PCR, the amount of product is proportional to the initial number of template copies.

If the amplification is performed in the presence of an internally quenched fluorescent oligonucleotide (TaqMan probe) complementary to the target sequence, the probe is cleaved by the 5'-3' endonuclease activity of Taq DNA polymerase and a fluorescent dye released in the medium (Holland et al.). Since the fluorescence emission will increase in direct proportion to the amount of the specific amplified product, the exponential growth phase of PCR product can be detected and used to determine the initial template concentration (Heid et al., 1996, and Gibson et al., 1996).

The amplification of an endogenous control can be performed to standardize the amount of sample RNA added to a reaction. In this kind of experiments the control of choice is the 18S ribosomal RNA. Since reporter dyes with differing emission spectra are available, the target and the endogenous control can be independently quantified in the same tube if probes labeled with different dyes are used.

All "real time PCR" measurements of fluorescence were made in the ABI Prism 7700 Sequence detector System (PE Applied Biosystems, Foster City, CA).

References

- Higuchi, R., Dollinger, G., Walsh, P.S. and Griffith, R. 1992. Simultaneous amplification and detection of specific DNA sequences. *BioTechnology* 10:413-417.

- Higuchi, R., Fockler, C., Dollinger, G. and Watson, R. 1993. Kinetic PCR analysis: real-time monitoring of DNA amplification reactions. . *BioTechnology* 11:1026-1030.

- Holland, P.M., Abramson, R.D., Watson, R. and Gelfand, D.H. 1991. Detection of specific polymerase chain reaction product by utilizing the 5'-3' exonuclease activity of *Thermus aquaticus* DNA polymerase. *Proc. Natl. Acad. Sci.* 88:7276-7280.

- Heid, C., Stevens, J., Livak, K. And Williams, P.M. 1996. Real time quantitative PCR. *Genome Res.* 6:986-994.

- Gibson, U.E., Heid, C.A. and Williams, P.M. 1996. A novel method for real time quantitative RT-PCR. *Genome Res.* 6: 995-1001.

cDNA preparation

The total RNAs used for expression quantification are listed in Table 3 along with their sources. Fifty µgs of each RNA were treated with DNase I for 1 hour at 37°C in the following reaction mix:

DNase I, RNase-free (Roche Diagnostics, Germany)	0.2 U/µL
RNase inhibitor (PE Applied Biosystems, CA)	0.4 U/µL
Tris-HCl pH 7.9	10mM
$MgCl_2$	10mM
NaCl	50mM
DTT	1mM

After incubation, RNA was extracted once with 1 volume of phenol:chloroform:isoamyl alcohol (24:24:1) and once with chloroform, and precipitated with 1/10 volume of NaAcetate 3M pH5.2 and 2 volume ethanol. After spectrophotometric quantification, each sample has been reverse transcribed with the TaqMan Reverse Transcription Reagents (PE Applied Biosystems, CA) accordingly to purchaser protocol. RNA final concentration in the reaction mix was 200ng/µL. Reverse transcription was made with 2.5µM of random hexamers.

TaqMan quantitative analysis

Specific primers and probe were designed accordingly to PE Applied Biosystems recommendations and are listed below:

forward primer: 5'-AACAGCACGCGCGAGAGT-3'

reverse primer: 5'-GCTGATGGGCATTTTGGAGA-3'

probe: 5'-(FAM) ACAGCAGCCACACGTGCATGCC (TAMRA)-3'

where FAM = 6-carboxy-fluorescein

and TAMRA = 6-carboxy-tetramethyl-rhodamine.

The expected length of the PCR product was 63bp.

Quantification experiments were performed on 50 ng of reverse transcribed RNA from each sample. Each determination was done in triplicate.

Total cDNA content was normalized with the simultaneous quantification (multiplex PCR) of the 18S ribosomal RNA by use of the Pre-Developed TaqMan Assay Reagents (PDAR) Control Kit (PE Applied Biosystems, CA).

Assay reaction mix was as follows:

	final
TaqMan Universal PCR Master Mix (2x) (PE Applied Biosystems, CA)	1x
PDAR control – 18S RNA (20x	1x
Forward primer	900nM
Reverse primer	300nM
Probe	200nM
cDNA	10ng
Water	to 25µL

PCR conditions were:

1 time the following steps:	
pre PCR	2' at 50°C
	10' at 95°C
40 times the following steps:	
denaturation	15'' at 95°C
annealing/extension	1' at 60°C

The experiment was performed on an ABI Prism 7700 Sequence Detector (PE Applied Biosystems, CA). At the end of the run, fluorescence data acquired during PCR were processed as described in the ABI Prism 7700 user's manual in order to achieve better background subtraction as well as signal linearity with the starting target quantity.

Results are shown in Fig. 10, 11 and 12.

Table 1 (see Fig. 10)

RNA	Purch.&#catalog
h. Fetal Brain	Clontech (CA) 640191
h. Brain	OriGene (MD) HT1001
h. Muscle	OriGene (MD) HT1008
h. Heart	OriGene (MD) HT1002
h. Lung	OriGene (MD) HT1009
h. Kidney	OriGene (MD) HT1003
h. Liver	OriGene (MD) HT1005
h. Thymus	Clontech (CA) 640281
h. Testis	OriGene (MD) HT1011
h. Colon	OriGene (MD) HT1015
h. Placenta	OriGene (MD) HT1013

Table 2 (see Fig. 11)

RNA	Purch.&#catalog
h. Fetal Liver	Clontech (CA) 640181
h. Bladder	Invitrogen (CA) D602001
h. Prostate	Clontech (CA) 640381
h. Adrenal Gland	Clontech (CA) 640161
h. Spleen	OriGene (MD) HT1004
h. Hypertrophic Prostate	from autopsy
h. Prostate	from autopsy

Table 3

cDNA	Description	Catalog
H. Brain whole	from 2 female, years 16 and 36	7187-1
H. Brain, cerebral cortex	from 1 male, 66 years.	7110-1
H. Brain, cerebellum	from 11 male/female, 16-70 years.	7120-1
H. Brain, hippocampus	From 25 male/female, 16-70 years.	7169-1
H. Spinal Cord	from 69 male/female, years. 22-70	7163-1
H. Heart	from 7 male/female, years. 20-78	7121-1
H. Kidney	from 8 male/female, years. 24-55	7112-1
H. Liver	from 1 male/1 female, years. 44 and 45	7113-1
Fetal Liver		640181
H.Bladder		D602001 Invitrogen
cDNA	**Description**	**Catalog**
H. Prostate		640381
H. Adrenal gland		640161
H. Spleen		HT1004 Origene

CLAIMS

1. An isolated polynucleotide encoding a α_{1a} adrenergic receptor-like GPCR polypeptide and being selected from the group consisting of:

a) a polynucleotide encoding a α_{1a} adrenergic receptor-like GPCR polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO. 3; and
the amino acid sequence shown in SEQ ID NO. 3.

b) a polynucleotide comprising the sequence of SEQ ID NO. 2;

c) a polynucleotide which hybridizes under stringent conditions to a polynucleotide specified in (a) and (b);

d) a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code; and

e) a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a) to (d).

2. An expression vector containing any polynucleotide of claim 1.

3. A host cell containing the expression vector of claim 2.

4. A substantially purified α_{1a} adrenergic receptor-like GPCR polypeptide encoded by a polynucleotide of claim 1.

5. A method for producing a α_{1a} adrenergic receptor-like GPCR polypeptide, wherein the method comprises the following steps:

a) culturing the host cell of claim 3 under conditions suitable for the expression of the α_{1a} adrenergic receptor-like GPCR polypeptide; and

b) recovering the α_{1a} adrenergic receptor-like GPCR polypeptide from the host cell culture.

6. A method for detection of a polynucleotide encoding a α_{1a} adrenergic receptor-like GPCR polypetide in a biological sample comprising the following steps:

a) hybridizing any polynucleotide of claim 1 to a nucleic acid material of a biological sample, thereby forming a hybridization complex; and

b) detecting said hybridization complex.

7. The method of claim 6, wherein before hybridization, the nucleic acid material of the biological sample is amplified.

8. A method for the detection of a polynucleotide of claim 1 or a α_{1a} adrenergic receptor-like GPCR polypeptide of claim 4 comprising the steps of:

contacting a biological sample with a reagent which specifically interacts with the polynucleotide or the α_{1a} adrenergic receptor-like GPCR polypeptide.

9. A diagnostic kit for conducting the method of any one of claims 6 to 8.

10. A method of screening for agents which decrease the activity of a α_{1a} adrenergic receptor-like GPCR, comprising the steps of:

contacting a test compound with any α_{1a} adrenergic receptor-like GPCR polypeptide encoded by any polynucleotide of claim 1;

detecting binding of the test compound to the α_{1a} adrenergic receptor-like GPCR polypeptide, wherein a test compound which binds to the polypeptide is identified as a potential therapeutic agent for decreasing the activity of a α_{1a} adrenergic receptor-like GPCR.

11. A method of screening for agents which regulate the activity of a α_{1a} adrenergic receptor-like GPCR, comprising the steps of:

contacting a test compound with a α_{1a} adrenergic receptor-like GPCR polypeptide encoded by any polynucleotide of claim 1; and

detecting a α_{1a} adrenergic receptor-like GPCR activity of the polypeptide, wherein a test compound which increases the α_{1a} adrenergic receptor-like GPCR activity is identified as a potential therapeutic agent for increasing the activity of the α_{1a} adrenergic receptor-like GPCR, and wherein a test compound which decreases the α_{1a} adrenergic receptor-like GPCR activity of the polypeptide is identified as a potential therapeutic agent for decreasing the activity of the α_{1a} adrenergic receptor-like GPCR.

12. A method of screening for agents which decrease the activity of a α_{1a} adrenergic receptor-like GPCR, comprising the steps of:

contacting a test compound with any polynucleotide of claim 1 and

detecting binding of the test compound to the polynucleotide, wherein a test compound which binds to the polynucleotide is identified as a potential therapeutic agent for decreasing the activity of α_{1a} adrenergic receptor-like GPCR.

13. A method of reducing the activity of α_{1a} adrenergic receptor-like GPCR, comprising the steps of:

contacting a cell with a reagent which specifically binds to any polynucleotide of claim 1 or any α_{1a} adrenergic receptor-like GPCR polypeptide of claim 4, whereby the activity of α_{1a} adrenergic receptor-like GPCR is reduced.

14. A reagent that modulates the activity of a α_{1a} adrenergic receptor-like GPCR polypeptide or a polynucleotide wherein said reagent is identified by the method of any of the claims 10 to 12.

15. A pharmaceutical composition, comprising:

the expression vector of claim 2 or the reagent of claim 14 and a pharmaceutically acceptable carrier.

16. Use of the pharmaceutical composition of claim 15 for modulating the activity of a α_{1a} adrenergic receptor-like GPCR in a disease.

17. Use of claim 16 wherein the disease is peripheral or central nervous system disease, urinary incontinence or benign prostatic hypertrophy.

18. A cDNA encoding a polypeptide comprising the amino acid sequence shown in SEQ ID NO. 3.

19. The cDNA of claim 18 which comprises SEQ ID NO. 2.

20. The cDNA of claim 18 which consists of SEQ ID NO. 2.

21. An expression vector comprising a polynucleotide which encodes a polypeptide comprising the amino acid sequence shown in SEQ ID NO. 3.

22. The expression vector of claim 21 wherein the polynucleotide consists of SEQ ID NO. 2.

23. A host cell comprising an expression vector which encodes a polypeptide comprising the amino acid sequence shown in SEQ ID NO. 3.

24. The host cell of claim 23 wherein the polynucleotide consists of SEQ ID NO. 2.

25. A purified polypeptide comprising the amino acid sequence shown in SEQ ID NO. 3.

26. The purified polypeptide of claim 25 which consists of the amino acid sequence shown in SEQ ID NO. 3.

27. A fusion protein comprising a polypeptide having the amino acid sequence shown in SEQ ID NO. 3.

28. A method of producing a polypeptide comprising the amino acid sequence shown in SEQ ID NO. 3, comprising the steps of:

culturing a host cell comprising an expression vector which encodes the polypeptide under conditions whereby the polypeptide is expressed; and isolating the polypeptide.

29. The method of claim 28 wherein the expression vector comprises SEQ ID NO. 2.

30. A method of detecting a coding sequence for a polypeptide comprising the amino acid sequence shown in SEQ ID NO. 3, comprising the steps of:

hybridizing a polynucleotide comprising 11 contiguous nucleotides of SEQ ID NO. 2 to nucleic acid material of a biological sample, thereby forming a hybridization complex; and detecting the hybridization complex.

31. The method of claim 30 further comprising the step of amplifying the nucleic acid material before the step of hybridizing.

32. A kit for detecting a coding sequence for a polypeptide comprising the amino acid sequence shown in SEQ ID NO. 3, comprising:

a polynucleotide comprising 11 contiguous nucleotides of SEQ ID NO. 2; and instructions for the method of claim 30.

33. A method of detecting a polypeptide comprising the amino acid sequence shown in SEQ ID NO. 3, comprising the steps of:

contacting a biological sample with a reagent that specifically binds to the polypeptide to form a reagent-polypeptide complex; and detecting the reagent-polypeptide complex.

34. The method of claim 33 wherein the reagent is an antibody.

35. A kit for detecting a polypeptide comprising the amino acid sequence shown in SEQ ID NO. 3, comprising:

an antibody which specifically binds to the polypeptide; and

instructions for the method of claim 33.

36. A method of screening for agents which can regulate the activity of a α_{1a} adrenergic receptor-like GPCR protein, comprising the steps of:

contacting a test compound with a polypeptide comprising an amino acid sequence selected from the group consisting of: (1) amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO. 3 and (2) the amino acid sequence shown in SEQ ID NO. 3; and

detecting binding of the test compound to the polypeptide, wherein a test compound which binds to the polypeptide is identified as a potential agent for regulating activity of the α_{1a} adrenergic receptor-like GPCR protein.

37. The method of claim 36 wherein the step of contacting is in a cell.

38. The method of claim 36 wherein the cell is *in vitro*.

39. The method of claim 36 wherein the step of contacting is in a cell-free system.

40. The method of claim 36 wherein the polypeptide comprises a detectable label.

41. The method of claim 36 wherein the test compound comprises a detectable label.

42. The method of claim 36 wherein the test compound displaces a labeled ligand which is bound to the polypeptide.

43. The method of claim 36 wherein the polypeptide is bound to a solid support.

44. The method of claim 36 wherein the test compound is bound to a solid support.

45. A method of screening for agents which regulate an activity of a human human α_{1a} adrenergic receptor-like GPCR protein, comprising the steps of:

contacting a test compound with a polypeptide comprising an amino acid sequence selected from the group consisting of: (1) amino acid sequences which are at least about 50% identical to the amino acid sequence shown in SEQ ID NO. 3 and (2) the amino acid sequence shown in SEQ ID NO. 3; and

detecting an activity of the polypeptide, wherein a test compound which increases the activity of the polypeptide is identified as a potential agent for increasing the activity of the human α_{1a} adrenergic receptor-like GPCR protein, and wherein a test compound which decreases the activity of the polypeptide is identified as a potential agent for decreasing the activity of the human α_{1a} adrenergic receptor-like GPCR protein.

46. The method of claim 45 wherein the step of contacting is in a cell.

47. The method of claim 45 wherein the cell is *in vitro*.

48. The method of claim 45 wherein the step of contacting is in a cell-free system.

49. The method of claim 45 wherein the activity is cyclic AMP formation.

50. The method of claim 45 wherein the activity is mobilization of intracellular calcium.

51. The method of claim 45 wherein the activity is phosphoinositide metabolism.

52. A method of screening for agents which regulate an activity of a human α_{1a} adrenergic receptor-like GPCR protein, comprising the steps of:

contacting a test compound with a product encoded by a polynucleotide which comprises the nucleotide sequence shown in SEQ ID NO. 2; and

detecting binding of the test compound to the product, wherein a test compound which binds to the product is identified as a potential agent for regulating the activity of the human α_{1a} adrenergic receptor-like GPCR protein.

53. The method of claim 52 wherein the product is a polypeptide.

54. The method of claim 52 wherein the product is RNA.

55. A method of reducing activity of a human α_{1a} adrenergic receptor-like GPCR protein, comprising the step of:

contacting a cell with a reagent which specifically binds to a product encoded by a polynucleotide comprising the nucleotide sequence shown in SEQ ID NO. 2, whereby the activity of a human α_{1a} adrenergic receptor-like GPCR protein is reduced.

56. The method of claim 55 wherein the product is a polypeptide.

57. The method of claim 56 wherein the reagent is an antibody.

58. The method of claim 55 wherein the product is RNA.

59. The method of claim 58 wherein the reagent is an antisense oligonucleotide.

60. The method of claim 58 wherein the reagent is a ribozyme.

61. The method of claim 55 wherein the cell is *in vitro*.

62. The method of claim 55 wherein the cell is *in vivo*.

63. A pharmaceutical composition, comprising:

a reagent which specifically binds to a polypeptide comprising the amino acid sequence shown in SEQ ID NO. 3; and

a pharmaceutically acceptable carrier.

64. The pharmaceutical composition of claim 63 wherein the reagent is an antibody.

65. A pharmaceutical composition, comprising:

a reagent which specifically binds to a product of a polynucleotide comprising the nucleotide sequence shown in SEQ ID NO. 2; and

a pharmaceutically acceptable carrier.

66. The pharmaceutical composition of claim 65 wherein the reagent is a ribozyme.

67. The pharmaceutical composition of claim 65 wherein the reagent is an antisense oligonucleotide.

68. The pharmaceutical composition of claim 65 wherein the reagent is an antibody.

69. A pharmaceutical composition, comprising:

an expression vector encoding a polypeptide comprising the amino acid sequence shown in SEQ ID NO. 3; and

a pharmaceutically acceptable carrier.

70. The pharmaceutical composition of claim 69 wherein the expression vector comprises SEQ ID NO. 2.

71. A method of treating α_{1a} adrenergic receptor-like GPCR, comprising the step of:,

administering to a patient in need thereof a therapeutically effective dose of a reagent that inhibits a function of a human α_{1a} adrenergic receptor-like GPCR protein, whereby symptoms of the α_{1a} adrenergic receptor-like GPCR are ameliorated.

72. The method of claim 71 wherein the reagent is identified by the method of claim 36.

73. The method of claim 71 wherein the reagent is identified by the method of claim 45.

74. The method of claim 71 wherein the reagent is identified by the method of claim 52.

75. A method of treating a α_{1a} adrenergic receptor-like GPCR disorder, comprising the step of:

administering to a patient in need thereof a therapeutically effective dose of a reagent that inhibits a function of a human α_{1a} adrenergic receptor-like GPCR protein, whereby symptoms of the α_{1a} adrenergic receptor-like GPCR disorder are ameliorated.

76. The method of claim 75 wherein the reagent is identified by the method of claim 36.

77. The method of claim 75 wherein the reagent is identified by the method of claim 45.

78. The method of claim 75 wherein the reagent is identified by the method of claim 52.

79. The method of claim 75 wherein the α_{1a} adrenergic receptor-like GPCR disorder is peripheral or central nervous system disease, urinary incontince or benign prostatic hyperplasia.

Fig. 1

AAAGGAGCCATAGAAGCTGCCCGCACTGGGGATGGAGCCGTGCGGAAAC
CCGGGGTAGGGGGTCCTGCAGCGTCCTTGCTGGGCGCGGAGGCTTCTCC
CCTTGACGGGTGACTAACTCTGCCTGCGTGTTTCTTTTGTCACCAGCAT
AGGCACTGAGTGCGGTCTGTGCACCCCTTTGCCACCCACCGGTGCCGGC
ACTGAGCCTGCAACCTGTCTCACGCCCTCTGGCTGTTGCCATGACGTC
CACCTGCACCAACAGCACGCGCGAGAGTAACAGCAGCCACACGTGCATG
CCCCTCTCCAAAATGCCCATCAGCCTGGCCCACGGCATCATCCGCTCAA
CCGTGCTGGTTATCTTCCTCGCCGCCTCTTTCGTCGGCAACATAGTGCT
GGCGCTAGTGTTGCAGCGCAAGCCGCAGCTGCTGCAGGTGACCAACCGT
TTTATCTTTAACCTCCTCGTCACCGACCTGCTGCAGATTTCGCTCGTGG
CCCCCTGGGTGGTGGCCACCTCTGTGCCTCTCTTCTGGCCCCTCAACAG
CCACTTCTGCACGGCCCTGGTTAGCCTCACCCACCTGTTCGCCTTCGCC
AGCGTCAACACCATTGTCGTGGTGTCAGTGGATCGCTACTTGTCCATCA
TCCACCCTCTCTCCTACCCGTCCAAGATGACCCAGCGCCGCGGTTACCT
GCTCCTCTATGGCACCTGGATTGTGGCCATCCTGCAGAGCACTCCTCCA
CTCTACGGCTGGGGCCAGGCTGCCTTTGATGAGCGCAATGCTCTCTGCT
CCATGATCTGGGGGGCCAGCCCCAGCTACACTATTCTCAGCGTGGTGTC
CTTCATCGTCATTCCACTGATTGTCATGATTGCCTGCTACTCCGTGGTG
TTCTGTGCAGCCCGGAGGCAGCATGCTCTGCTGTACAATGTCAAGAGAC
ACAGCTTGGAAGTGCGAGTCAAGGACTGTGTGGAGAATGAGGATGAAGA
GGGAGCAGAGAAGAAGGAGGAGTTCCAGGATGAGAGTGAGTTTCGCCGC
CAGCATGAAGGTGAGGTCAAGGCCAAGGAGGGCAGAATGGAAGCCAAGG
ACGGCAGCCTGAAGGCCAAGGAAGGAAGCACGGGGACCAGTGAGAGTAG
TGTAGAGGCCAGGGGCAGCGAGGAGGTCAGAGAGAGCAGCACGGTGGCC
AGCGACGGCAGCATGGAGGGTAAGGAAGGCAGCACCAAAGTTGAGGAGA
ACAGCATGAAGGCAGACAAGGGTCGCACAGAGGTCAACCAGTGCAGCAT
TGACTTGGGTGAAGATGACATGGAGTTTGGTGAAGACGACATCAATTTC
AGTGAGGATGACGTCGAGGCAGTGAACATCCCGGAGAGCCTCCCACCCA
GTCGTCGTAACAGCAACAGCAACCCTCCTCTGCCCAGGTGCTACCAGTG
CAAAGCTGCTAAAGTGATCTTCATCATCATTTTCTCCTATGTGCTATCC
CTGGGGCCCTACTGCTTTTTAGCAGTCCTGGCCGTGTGGGTGGATGTCG
AAACCCAGGTACCCCAGTGGGTGATCACCATAATCATCTGGCTTTTCTT
CCTGCAGTGCTGCATCCACCCCTATGTCTATGGCTACATGCACAAGACC
ATTAAGAAGGAAATCCAGGACATGCTGAAGAAGTTCTTCTGCAAGGAAA

AGCCCCCGAAAGAAGATAGCCACCCAGACCTGCCCGGAACAGAGGGTGG
GACTGAAGGCAAGATTGTCCCTTCCTACGATTCTGCTACTTTTCCTTG
AAGTTAGTTCTAAGGCAAACCTTGAACTGTCCATAACACGAGAAACAAG
AGGAGATTTCTTTTCAATGGACCCACAATTCATTAATGCCAAACCATAC
CATTTCAGGCAAAGGTGTTGCACACACATGCTCTTCACCACAAGGTAGA
TAAATATATAG

Fig. 2

ATGACGTCCACCTGCACCAACAGCACGCGCGAGAGTAACAGCAGCCACA
CGTGCATGCCCCTCTCCAAAATGCCCATCAGCCTGGCCCACGGCATCAT
CCGCTCAACCGTGCTGGTTATCTTCCTCGCCGCCTCTTTCGTCGGCAAC
ATAGTGCTGGCGCTAGTGTTGCAGCGCAAGCCGCAGCTGCTGCAGGTGA
CCAACCGTTTTATCTTTAACCTCCTCGTCACCGACCTGCTGCAGATTTC
GCTCGTGGCCCCCTGGGTGGTGGCCACCTCTGTGCCTCTCTTCTGGCCC
CTCAACAGCCACTTCTGCACGGCCCTGGTTAGCCTCACCCACCTGTTCG
CCTTCGCCAGCGTCAACACCATTGTCGTGGTGTCAGTGGATCGCTACTT
GTCCATCATCCACCCTCTCTCCTACCCGTCCAAGATGACCCAGCGCCGC
GGTTACCTGCTCCTCTATGGCACCTGGATTGTGGCCATCCTGCAGAGCA
CTCCTCCACTCTACGGCTGGGGCCAGGCTGCCTTTGATGAGCGCAATGC
TCTCTGCTCCATGATCTGGGGGGCCAGCCCCAGCTACACTATTCTCAGC
GTGGTGTCCTTCATCGTCATTCCACTGATTGTCATGATTGCCTGCTACT
CCGTGGTGTTCTGTGCAGCCCGGAGGCAGCATGCTCTGCTGTACAATGT
CAAGAGACACAGCTTGGAAGTGCGAGTCAAGGACTGTGTGGAGAATGAG
GATGAAGAGGGAGCAGAGAAGAAGGAGGAGTTCCAGGATGAGAGTGAGT
TTCGCCGCCAGCATGAAGGTGAGGTCAAGGCCAAGGAGGGCAGAATGGA
AGCCAAGGACGGCAGCCTGAAGGCCAAGGAAGGAAGCACGGGGACCAGT
GAGAGTAGTGTAGAGGCCAGGGGCAGCGAGGAGGTCAGAGAGAGCAGCA
CGGTGGCCAGCGACGGCAGCATGGAGGGTAAGGAAGGCAGCACCAAAGT
TGAGGAGAACAGCATGAAGGCAGACAAGGGTCGCACAGAGGTCAACCAG
TGCAGCATTGACTTGGGTGAAGATGACATGGAGTTTGGTGAAGACGACA
TCAATTTCAGTGAGGATGACGTCGAGGCAGTGAACATCCCGGAGAGCCT
CCCACCCAGTCGTCGTAACAGCAACAGCAACCCTCCTCTGCCCAGGTGC
TACCAGTGCAAAGCTGCTAAAGTGATCTTCATCATCATTTTCTCCTATG
TGCTATCCCTGGGGCCCTACTGCTTTTTAGCAGTCCTGGCCGTGTGGGT

GGATGTCGAAACCCAGGTACCCCAGTGGGTGATCACCATAATCATCTGG
CTTTTCTTCCTGCAGTGCTGCATCCACCCCTATGTCTATGGCTACATGC
ACAAGACCATTAAGAAGGAAATCCAGGACATGCTGAAGAAGTTCTTCTG
CAAGGAAAAGCCCCCGAAAGAAGATAGCCACCCAGACCTGCCCGGAACA
GAGGGTGGGACTGAAGGCAAGATTGTCCCTTCCTACGATTCTGCTACTT
TTCCTTGA

Fig. 3

MTSTCTNSTRESNSSHTCMPLSKMPISLAHGIIRSTVLVIFLAASFVGN
IVLALVLQRKPQLLQVTNRFIFNLLVTDLLQISLVAPWVVATSVPLFWP
LNSHFCTALVSLTHLFAFASVNTIVVVSVDRYLSIIHPLSYPSKMTQRR
GYRRGYLLLYGTWIVAILQSTPPLYGWGQAAFDERNALCSMIWGASPSY
TILSVVSFIVIPLIVMIACYSVVFCAARRQHALLYNVKRHSLEVRVKDC
VENEDEEGAEKKEEFQDESEFRRQHEGEVKAKEGRMEAKDGSLKAKEGS
TGTSESSVEARGSEEVRESSTVASDGSMEGKEGSTKVEENSMKADKGRT
EVNQCSIDLGEDDMEFGEDDINFSEDDVEAVNIPESLPPSRRNSNSNPP
LPRCYQCKAAKVIFIIIFSYVLSLGPYCFLAVLAVWVDVETQVPQWVIT
IIIWLFFLQCCIHPYVYGYMHKTIKKEIQDMLKKFFCKEKPPKEDSHPD
LPGTEGGTEGKIVPSYDSATFP

Fig. 4

MVFLSGNASD SSNCTHPPAP VNISKAILLG VILGGLILFG
VLGNILVILS VACHRHLHSV THYYIVNLAV ADLLLTSTVL
PFSAIFEILG YWAFGRVFCN IWAAVDVLCC TASIISLCVI
SIDRYIGVSY PLRYPTIVTQ RRGLRALLCV WAFSLVISVG
PLFGWRQPAP DDETICQINE EPGYVLFSAL GSFYVPLTII
LAMYCRVYVV AKRESRGLKS GLKTDKSDSE QVTLRIHRKN
APAGGSGVAS AKNKTHFSVR LLKFSREKKA AKTLGIVVGC
FVLCWLPFFL VMPIGSFFPD FKPPETVFKI VFWLGYLNSC
INPIIYPCSS QEFKKAFQNV LKIQCLRRKQ SSKHALGYTL
HAPSQALEGQ HKDMVRIPVG SGETFYKISK TDGVCEWKFF

SSMPRGSARI TVPKDQSACT TARVRSKSFL QVCCCVGPST
PNPGENHQVP TIKIHTISLS ENGEEV

Fig. 5

GGAGAGACAGAAACCACACGGAGAGACACAGAGACAAGAGGGTCCGAGAAAGAC
AGAGCAAGCGCACACGTGCCAGTGAGAGACAGAAGGAGACACCCAGAGAACCCG
AGGGAGACGCAGAAACAAGAGAGTTGGAGAGACACGGAGACGGGCAGAGACACA
GCGAACGACATTGAACAGGTGCGCTAGGGACCCACAAAGATGAACTGCTGGGGA
AATAGACTGAGAGAGAAATGGAGAGACAAGAGAGCACTAGGGAAAGACGGAGAC
ACACATGAACTAGCGAGCGACACAGTGACAGGCAGAGACACAGAGAGACAGAAT
GGATCAGAAAGAGACTGAGGGAGAGACGGGCAAGACTGTGAGAAAGATAGGGAG
CGAGAGCGCAAGCCCTGCAGAGAAAGTGCGCTAGCGAAAGAGACACACACAGAG
AGACTGAGTGACAAACAGAGTGGAAGAGATAAATGAGAGTAACAGGAAAGAGAC
AGCGTGCACGTGCTATCGAGAGACGGAGAAACAGAGACAGACATAAGTGAGAGA
CAAGGATGAGAAAGAATCAGGAAACGGTGAGACACATACAGACACACAACCCGA
GAGATAAAGAAAAAAAGTGCGCTAGCGAGAGACACTGGAGAAGAGACAGAAACT
GACGGTCGTAAACAGACAGAAACAGAGAGCGCACGCGCATGCGCTAGCGCTAGC
GACCAAAACTCCCCGGAGCCAGAGACAGTGTGAGAGACAAGCAGAGAAAGCGCA
CGCACGCACGCGCCAGCAGGAGAAACAGATGAGAGGAAATCAGAGCCCTGGAGA
GAGACAGGCAGACAGATCTGGAGAGTCCGGAAAGGAGCCATAGAAGCTGCCCGC
ACTGGGGATGGAGCCGTGCGGAAACCCGGGGTAGGGGGTCCTGCAGCGTCCTTG
CTGGGCGCGGAGGCTTCTCCCCTTGACGGGTGACTAACTCTGCCTGCGTGTTTC
TTTTGTCACCAGCATAGGCACTGAGTGCGGTCTGTGCACCCCTTTGCCACCCAC
CGGTGCCGGCACTGAGCCTGCAACCTGTCTCACGCCCTCTGGCTGTTGCCATGA
CGTCCACCTGCACCAACAGCACGCGCGAGAGTAACAGCAGCCACACGTGCATGC
CCCTCTCCAAAATGCCCATCAGCCTGGCCCACGGCATCATCCGCTCAACCGTGC
TGGTTA

Fig. 6

```
Q:   255 NSTRESNSSHTCMPLSKMPISLAHGIIRSTVLVIFLAASFVGNIVLALVLQRKPQLLQVT
         N:: .SN.:H.      ..P:::::..I: ..:L  .:. ..:GNI::.L :. . .L .VT
H:     7 NASDSSNCTHP-----PAPVNISKAILLGVILGGLILFGVLGNILVILSVACHRHLHSVT

         NRFIFNLLVTDLLQISLVAPWVVATSVPLFWPLNSHFCTALVSLTHLFAFASVNTIVVVS
         : :I.NL.V.DLL .S.V.P: .. .:  :W.... FC.. .::. L . AS: ::.V:S
         HYYIVNLAVADLLLTSTVLPFSAIFEILGYWAFGRVFCNIWAAVDVLCCTASIISLCVIS

         VDRYLSIIHPLSYPSKMTQRRGYLLLYGTWIVAILQSTPPLYGWGQAAFDERNALCSMIW
         :DRY:.: :PL.YP: :TQRRG. .L. .W.:::: S. PL:GW Q.A D: ..:C.:
         IDRYIGVSYPLRYPTIVTQRRGLRALLCVWAFSLVISVGPLFGWRQPAPDD-ETICQI--

           GASPSYTILSVVSFIVIPLIVMIACYSVVFCAARRQ     902
           ...P.Y.:.S.:. ..:PL.:::A.Y. V:..A:R:
           NEEPGYVLFSALGSFYVPLTIILAMYCRVYVVAKRE     214
```

Fig. 7

```
Q:  1422 KAAKVIFIIIFSYVLSLGPYCFLAVLAVWVDVETQVPQWVITIIIWLFFLQCCIHPYVYG
           KAAK.: I:: .:VL.  P: .:. :.. :.. : : P: V..I:.WL :L..CI:P..:Y
H:   269 KAAKTLGIVVGCFVLCWLPFFLVMPIGSFFP-DFKPPETVFKIVFWLGYLNSCINPIIYP

                 YMHKTIKKEIQDML    1643
                   ..:...KK..Q::L
                 CSSQEFKKAFQNVL     341
```

Fig. 8

HUMAN α_{1A} ADRENERGIC RECEPTOR-LIKE GPCR			
	RT-PCR		**RT-PCR**
Normal Tissue		**Cell lines**	
Adipose Sub.	+	1- Sk-Nr-Be (2)	-
Adipose Mes.	-	2-H4	+
Adrenal Gland	+	3-Sk-N-As	+
Bone Marrow	-	4-HTB-10	+
Brain	+	5-IMR-32	+
Colon	-	6-SNSY-5Y	-
Fetal brain	+	7-T3	+
Fetal liver	-	8-SK-N-D2	+
Heart	-	9-D283	+
Hypothalamus	+	10-DAOY	+
Kidney	-	11-CHP-212	+
Liver	+	12-U87MG	-
Lung	-	13-BE(2)C	-
Mammary Gland	+	14-T986	-
Pancreas	-	15-KANTS	+
Placenta	-	16-MO59K	+
Prostate	-	17-CHP234	-
Salivary Gland	-	18-C6 (RAT)	-

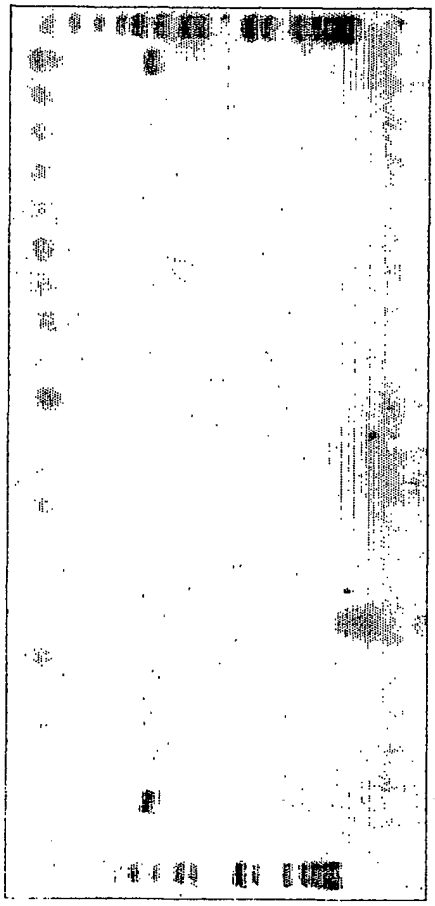
Skeletal Muscle	-	19-SK-N-F1	-
Small intestine	-	20-SK-PU-DW	-
Spleen	+		
Stomach	+		
Testes	+		
Thymus	+		
Thyroid	+		
Trachea	+		
Uterus	+		
Cortex	-		
Caudate	+		
Medulla	-		
Sub. Nigria	+/-		
Putamen	+		
cerebellum	+		
spinal cord	-		
Human Islets	+		

Fig. 9

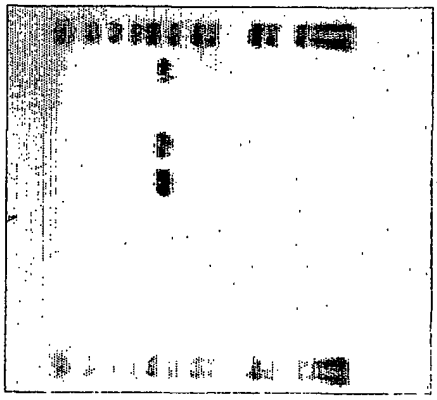
αA1 adrenergic GPCR

Phage libraries

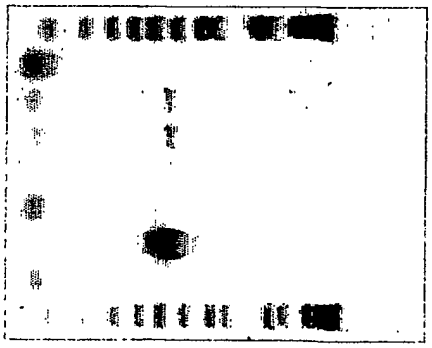
cDNA



mwm
1-Brain (c.str.)
2-Brain (fetal)
3-Brain (fr.cortex)
4-Brain (sub.nigr)
5-Brain (occ.cotx)
6-Brain Stem
7-Bronchial muscle
8-Coronary (endot.cells)
9-Coronary (sm.muscle cells)
10-Endothelial
11-Heart
12-Kidney
13-Liver
14-Lung
15-Sk Muscle
16-Ovary
17-Pulmunary artery
18-Umbelical artery
19- Whole Brain
20-Spinal cord
PC-Genomic DNA
NC-H2O
mwm

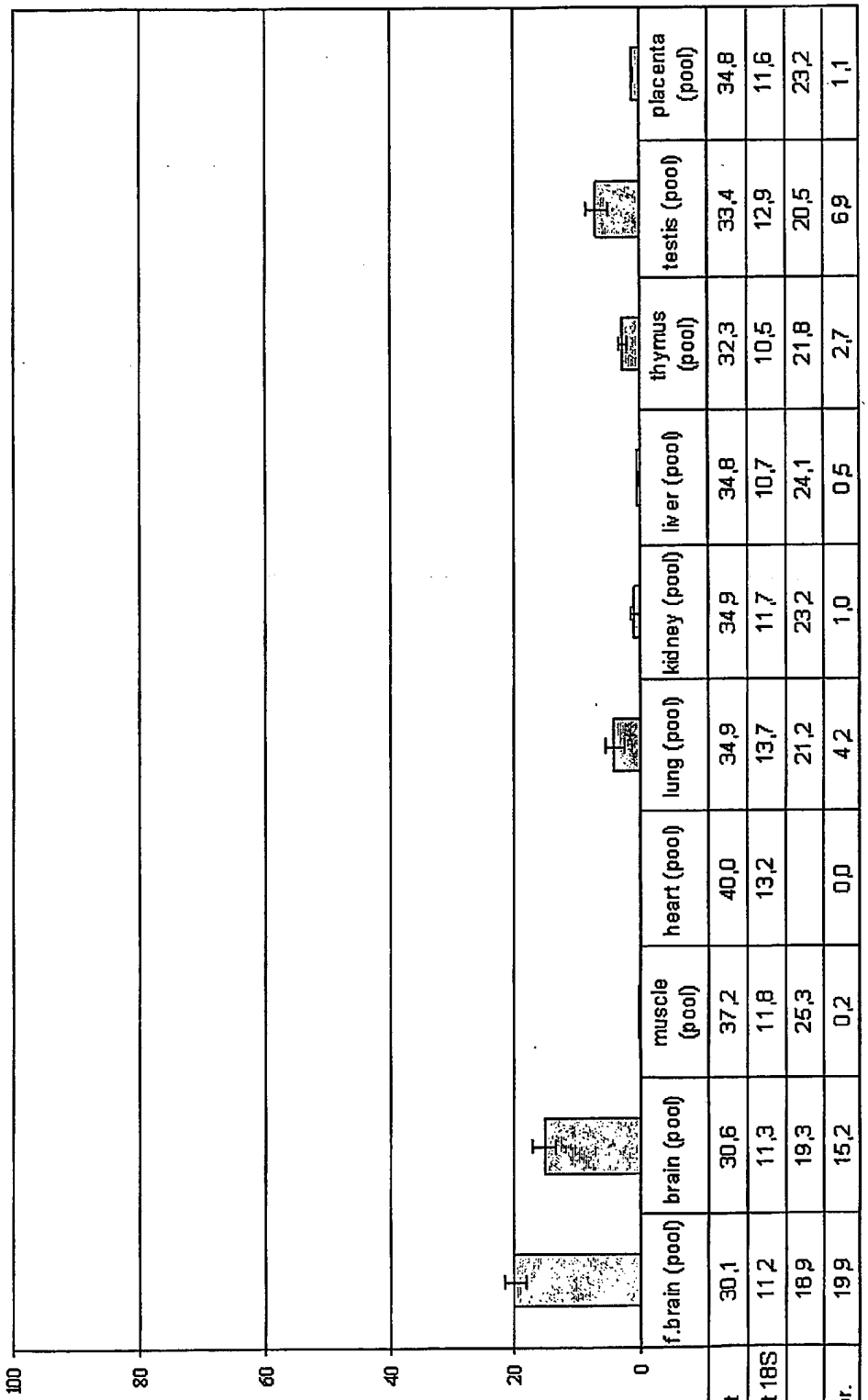


mwm
1-Whole Brain
2-Brain (cerebral cotx)
3-Brain (cerebellum)
4-Brain (hippocampus)
5-Spinal Cord
6-Heart
7-Kidney
8-Liver
mwm



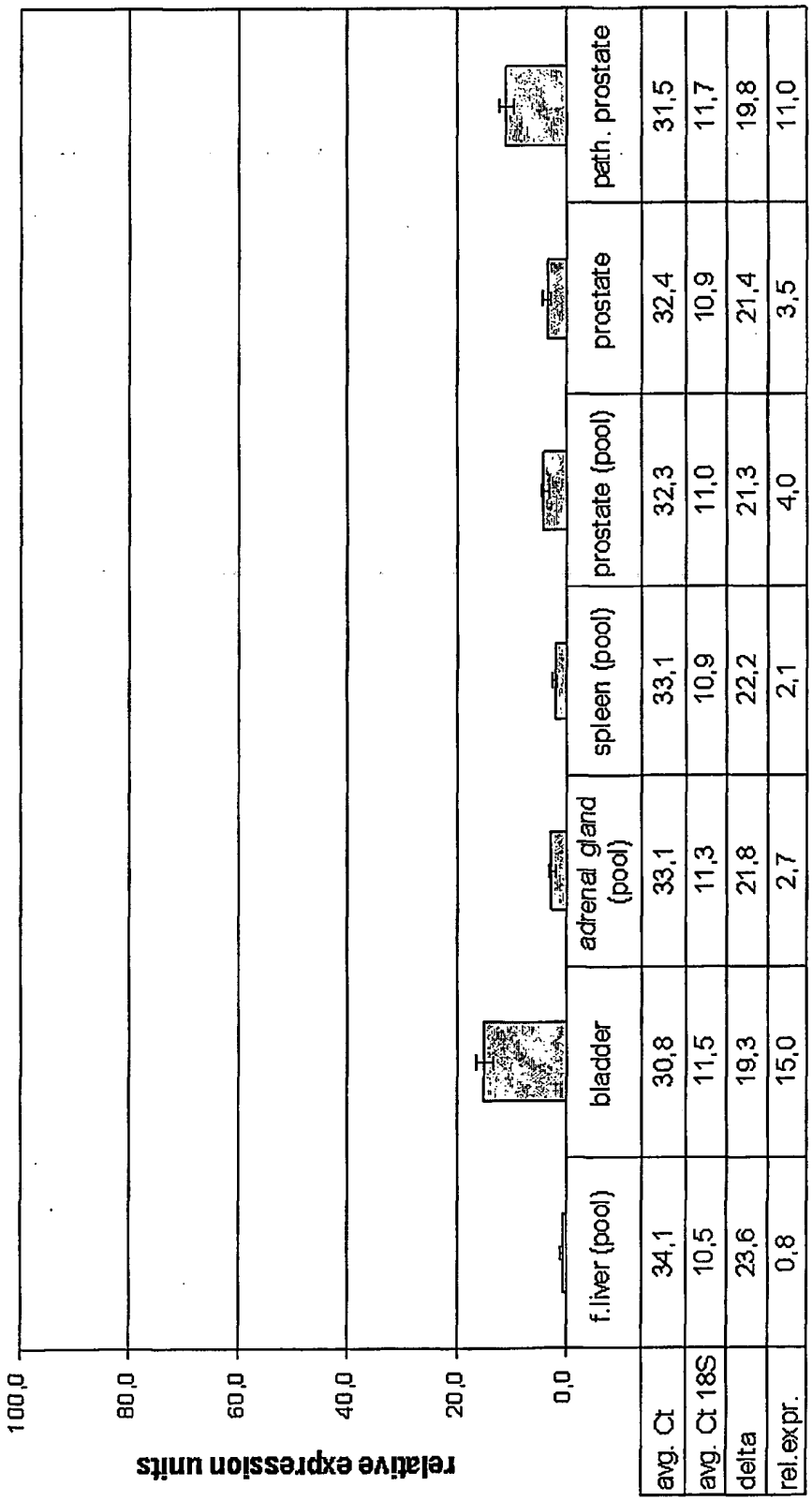
mwm
1-Fetal liver
2-Bladder
3-Prostate
4-Adrenal Gland
5-Spleen
PC-Genomic DNA
NC-H2O
mwm

Fig. 10



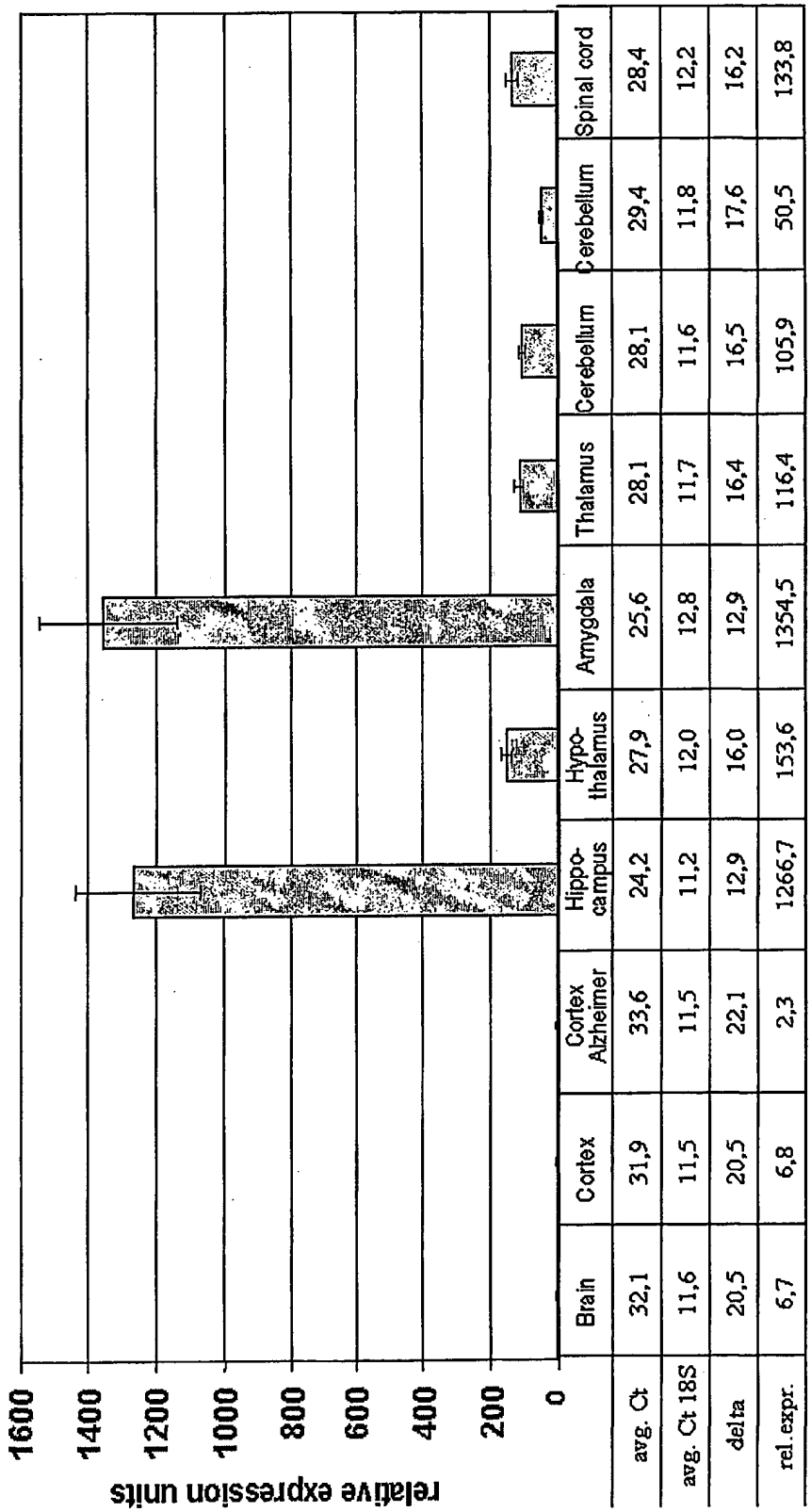
	f.brain (pool)	brain (pool)	muscle (pool)	heart (pool)	lung (pool)	kidney (pool)	liver (pool)	thymus (pool)	testis (pool)	placenta (pool)
avg. Ct	30,1	30,6	37,2	40,0	34,9	34,9	34,8	32,3	33,4	34,8
avg. Ct 18S	11,2	11,3	11,8	13,2	13,7	11,7	10,7	10,5	12,9	11,6
delta	18,9	19,3	25,3		21,2	23,2	24,1	21,8	20,5	23,2
rel.expr.	19,9	15,2	0,2	0,0	4,2	1,0	0,5	2,7	6,9	1,1

Fig. 11



	f.liver (pool)	bladder	adrenal gland (pool)	spleen (pool)	prostate (pool)	prostate	path. prostate
avg. Ct	34,1	30,8	33,1	33,1	32,3	32,4	31,5
avg. Ct 18S	10,5	11,5	11,3	10,9	11,0	10,9	11,7
delta	23,6	19,3	21,8	22,2	21,3	21,4	19,8
rel.expr.	0,8	15,0	2,7	2,1	4,0	3,5	11,0

Fig. 12



	Brain	Cortex	Cortex Alzheimer	Hippo-campus	Hypo-thalamus	Amygdala	Thalamus	Cerebellum	Cerebellum	Spinal cord
avg. Ct	32,1	31,9	33,6	24,2	27,9	25,6	28,1	28,1	29,4	28,4
avg. Ct 18S	11,6	11,5	11,5	11,2	12,0	12,8	11,7	11,6	11,8	12,2
delta	20,5	20,5	22,1	12,9	16,0	12,9	16,4	16,5	17,6	16,2
rel. expr.	6,7	6,8	2,3	1266,7	153,6	1354,5	116,4	105,9	50,5	133,8

SEQUENCE LISTING

<110> Bayer AG

<120> REGULATION OF HUMAN alpha1A ADRENERGIC RECEPTOR-LIKE G PROTEIN-COUPLED RECEPTOR

<130> Lio062 Foreign Countries

<140>
<141>

<150> US 60/204,145
<151> 2000-05-15

<150> US 60/250,505
<151> 2000-12-04

<160> 5

<170> PatentIn Ver. 2.1

<210> 1
<211> 1920
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> (237)..(1763)
<223> coding sequence

<400> 1
aaaggagcca tagaagctgc ccgcactggg gatggagccg tgcggaaacc cggggtaggg 60
ggtcctgcag cgtccttgct gggcgcggag gcttctcccc ttgacgggtg actaactctg 120
cctgcgtgtt tcttttgtca ccagcatagg cactgagtgc ggtctgtgca ccccttgcc 180
acccaccggt gccggcactg agcctgcaac ctgtctcacg ccctctggct gttgccatga 240
cgtccacctg caccaacagc acgcgcgaga gtaacagcag ccacacgtgc atgcccctct 300
ccaaaatgcc catcagcctg gcccacggca tcatccgctc aaccgtgctg gttatcttcc 360
tcgccgcctc tttcgtcggc aacatagtgc tggcgctagt gttgcagcgc aagccgcagc 420
tgctgcaggt gaccaaccgt tttatcttta acctcctcgt caccgacctg ctgcagattt 480
cgctcgtggc cccctgggtg gtggccacct ctgtgcctct cttctggccc ctcaacagcc 540
acttctgcac ggccctggtt agcctcaccc acctgttcgc cttcgccagc gtcaacacca 600
ttgtcgtggt gtcagtggat cgctacttgt ccatcatcca ccctctctcc tacccgtcca 660
agatgaccca gcgccgcggt tacctgctcc tctatggcac ctggattgtg gccatcctgc 720
agagcactcc tccactctac ggctggggcc aggctgcctt tgatgagcgc aatgctctct 780
gctccatgat ctgggggggcc agccccagct acactattct cagcgtggtg tccttcatcg 840
tcattccact gattgtcatg attgcctgct actccgtggt gttctgtgca gcccggaggc 900

agcatgctct gctgtacaat gtcaagagac acagcttgga agtgcgagtc aaggactgtg 960
tggagaatga ggatgaagag ggagcagaga agaaggagga gttccaggat gagagtgagt 1020
ttcgccgcca gcatgaaggt gaggtcaagg ccaaggaggg cagaatggaa gccaaggacg 1080
gcagcctgaa ggccaaggaa ggaagcacgg ggaccagtga gagtagtgta gaggccaggg 1140
gcagcgagga ggtcagagag agcagcacgg tggccagcga cggcagcatg gagggtaagg 1200
aaggcagcac caaagttgag gagaacagca tgaaggcaga caagggtcgc acagaggtca 1260
accagtgcag cattgacttg ggtgaagatg acatggagtt tggtgaagac gacatcaatt 1320
tcagtgagga tgacgtcgag gcagtgaaca tcccggagag cctcccaccc agtcgtcgta 1380
acagcaacag caaccctcct ctgcccaggt gctaccagtg caaagctgct aaagtgatct 1440
tcatcatcat tttctcctat gtgctatccc tggggcccta ctgcttttta gcagtcctgg 1500
ccgtgtgggt ggatgtcgaa acccaggtac cccagtgggt gatcaccata atcatctggc 1560
ttttcttcct gcagtgctgc atccacccct atgtctatgg ctacatgcac aagaccatta 1620
agaaggaaat ccaggacatg ctgaagaagt tcttctgcaa ggaaaagccc ccgaaagaag 1680
atagccaccc agacctgccc ggaacagagg gtgggactga aggcaagatt gtcccttcct 1740
acgattctgc tacttttcct tgaagttagt tctaaggcaa accttgaact gtccataaca 1800
cgagaaacaa gaggagattt cttttcaatg gacccacaat tcattaatgc caaaccatac 1860
catttcaggc aaaggtgttg cacacacatg ctcttcacca caaggtagat aaatatatag 1920

<210> 2
<211> 1527
<212> DNA
<213> Homo sapiens

<400> 2
atgacgtcca cctgcaccaa cagcacgcgc gagagtaaca gcagccacac gtgcatgccc 60
ctctccaaaa tgcccatcag cctggcccac ggcatcatcc gctcaaccgt gctggttatc 120
ttcctcgccg cctctttcgt cggcaacata gtgctggcgc tagtgttgca gcgcaagccg 180
cagctgctgc aggtgaccaa ccgttttatc tttaacctcc tcgtcaccga cctgctgcag 240
atttcgctcg tggcccctg ggtggtggcc acctctgtgc ctctcttctg gcccctcaac 300
agccacttct gcacggccct ggttagcctc acccacctgt tcgccttcgc cagcgtcaac 360
accattgtcg tggtgtcagt ggatcgctac ttgtccatca tccaccctct ctcctacccg 420
tccaagatga cccagcgccg cggttacctg ctcctctatg gcacctggat tgtggccatc 480
ctgcagagca ctcctccact ctacggctgg ggccaggctg cctttgatga gcgcaatgct 540
ctctgctcca tgatctgggg ggccagcccc agctacacta ttctcagcgt ggtgtccttc 600
atcgtcattc cactgattgt catgattgcc tgctactccg tggtgttctg tgcagcccgg 660
aggcagcatg ctctgctgta caatgtcaag agacacagct tggaagtgcg agtcaaggac 720
tgtgtggaga atgaggatga agagggagca gagaagaagg aggagttcca ggatgagagt 780
gagtttcgcc gccagcatga aggtgaggtc aaggccaagg agggcagaat ggaagccaag 840
gacggcagcc tgaaggccaa ggaaggaagc acggggacca gtgagagtag tgtagaggcc 900
aggggcagcg aggaggtcag agagagcagc acggtggcca gcgacggcag catggagggt 960
aaggaaggca gcaccaaagt tgaggagaac agcatgaagg cagacaaggg tcgcacagag 1020
gtcaaccagt gcagcattga cttgggtgaa gatgacatgg agtttggtga agacgacatc 1080
aatttcagtg aggatgacgt cgaggcagtg aacatcccgg agagcctccc acccagtcgt 1140
cgtaacagca acagcaaccc tcctctgccc aggtgctacc agtgcaaagc tgctaaagtg 1200
atcttcatca tcattttctc ctatgtgcta tccctggggc cctactgctt tttagcagtc 1260
ctggccgtgt gggtggatgt cgaaacccag gtaccccagt gggtgatcac cataatcatc 1320
tggcttttct tcctgcagtg ctgcatccac ccctatgtct atggctacat gcacaagacc 1380

attaagaagg aaatccagga catgctgaag aagttcttct gcaaggaaaa gcccccgaaa 1440
gaagatagcc acccagacct gcccggaaca gagggtggga ctgaaggcaa gattgtccct 1500
tcctacgatt ctgctacttt tccttga 1527

<210> 3
<211> 508
<212> PRT
<213> Homo sapiens

<400> 3

```
Met Thr Ser Thr Cys Thr Asn Ser Thr Arg Glu Ser Asn Ser Ser His
  1               5                  10                  15

Thr Cys Met Pro Leu Ser Lys Met Pro Ile Ser Leu Ala His Gly Ile
             20                  25                  30

Ile Arg Ser Thr Val Leu Val Ile Phe Leu Ala Ala Ser Phe Val Gly
         35                  40                  45

Asn Ile Val Leu Ala Leu Val Leu Gln Arg Lys Pro Gln Leu Leu Gln
     50                  55                  60

Val Thr Asn Arg Phe Ile Phe Asn Leu Leu Val Thr Asp Leu Leu Gln
 65                  70                  75                  80

Ile Ser Leu Val Ala Pro Trp Val Val Ala Thr Ser Val Pro Leu Phe
                 85                  90                  95

Trp Pro Leu Asn Ser His Phe Cys Thr Ala Leu Val Ser Leu Thr His
            100                 105                 110

Leu Phe Ala Phe Ala Ser Val Asn Thr Ile Val Val Val Ser Val Asp
        115                 120                 125

Arg Tyr Leu Ser Ile Ile His Pro Leu Ser Tyr Pro Ser Lys Met Thr
    130                 135                 140

Gln Arg Arg Gly Tyr Leu Leu Leu Tyr Gly Thr Trp Ile Val Ala Ile
145                 150                 155                 160

Leu Gln Ser Thr Pro Pro Leu Tyr Gly Trp Gly Gln Ala Ala Phe Asp
                165                 170                 175

Glu Arg Asn Ala Leu Cys Ser Met Ile Trp Gly Ala Ser Pro Ser Tyr
            180                 185                 190

Thr Ile Leu Ser Val Val Ser Phe Ile Val Ile Pro Leu Ile Val Met
```

195 200 205

Ile Ala Cys Tyr Ser Val Val Phe Cys Ala Ala Arg Arg Gln His Ala
210 215 220

Leu Leu Tyr Asn Val Lys Arg His Ser Leu Glu Val Arg Val Lys Asp
225 230 235 240

Cys Val Glu Asn Glu Asp Glu Glu Gly Ala Glu Lys Lys Glu Glu Phe
245 250 255

Gln Asp Glu Ser Glu Phe Arg Arg Gln His Glu Gly Glu Val Lys Ala
260 265 270

Lys Glu Gly Arg Met Glu Ala Lys Asp Gly Ser Leu Lys Ala Lys Glu
275 280 285

Gly Ser Thr Gly Thr Ser Glu Ser Ser Val Glu Ala Arg Gly Ser Glu
290 295 300

Glu Val Arg Glu Ser Ser Thr Val Ala Ser Asp Gly Ser Met Glu Gly
305 310 315 320

Lys Glu Gly Ser Thr Lys Val Glu Glu Asn Ser Met Lys Ala Asp Lys
325 330 335

Gly Arg Thr Glu Val Asn Gln Cys Ser Ile Asp Leu Gly Glu Asp Asp
340 345 350

Met Glu Phe Gly Glu Asp Asp Ile Asn Phe Ser Glu Asp Asp Val Glu
355 360 365

Ala Val Asn Ile Pro Glu Ser Leu Pro Pro Ser Arg Arg Asn Ser Asn
370 375 380

Ser Asn Pro Pro Leu Pro Arg Cys Tyr Gln Cys Lys Ala Ala Lys Val
385 390 395 400

Ile Phe Ile Ile Ile Phe Ser Tyr Val Leu Ser Leu Gly Pro Tyr Cys
405 410 415

Phe Leu Ala Val Leu Ala Val Trp Val Asp Val Glu Thr Gln Val Pro
420 425 430

Gln Trp Val Ile Thr Ile Ile Ile Trp Leu Phe Phe Leu Gln Cys Cys
435 440 445

Ile His Pro Tyr Val Tyr Gly Tyr Met His Lys Thr Ile Lys Lys Glu

```
    450                 455                 460

Ile Gln Asp Met Leu Lys Lys Phe Phe Cys Lys Glu Lys Pro Pro Lys
465                 470                 475                 480

Glu Asp Ser His Pro Asp Leu Pro Gly Thr Glu Gly Gly Thr Glu Gly
                485                 490                 495

Lys Ile Val Pro Ser Tyr Asp Ser Ala Thr Phe Pro
            500                 505
```

<210> 4
<211> 466
<212> PRT
<213> Homo sapiens

<400> 4

```
Met Val Phe Leu Ser Gly Asn Ala Ser Asp Ser Ser Asn Cys Thr His
  1               5                  10                  15

Pro Pro Ala Pro Val Asn Ile Ser Lys Ala Ile Leu Leu Gly Val Ile
            20                  25                  30

Leu Gly Gly Leu Ile Leu Phe Gly Val Leu Gly Asn Ile Leu Val Ile
        35                  40                  45

Leu Ser Val Ala Cys His Arg His Leu His Ser Val Thr His Tyr Tyr
    50                  55                  60

Ile Val Asn Leu Ala Val Ala Asp Leu Leu Leu Thr Ser Thr Val Leu
65                  70                  75                  80

Pro Phe Ser Ala Ile Phe Glu Ile Leu Gly Tyr Trp Ala Phe Gly Arg
                85                  90                  95

Val Phe Cys Asn Ile Trp Ala Ala Val Asp Val Leu Cys Cys Thr Ala
            100                 105                 110

Ser Ile Ile Ser Leu Cys Val Ile Ser Ile Asp Arg Tyr Ile Gly Val
        115                 120                 125

Ser Tyr Pro Leu Arg Tyr Pro Thr Ile Val Thr Gln Arg Arg Gly Leu
    130                 135                 140

Arg Ala Leu Leu Cys Val Trp Ala Phe Ser Leu Val Ile Ser Val Gly
145                 150                 155                 160
```

```
Pro Leu Phe Gly Trp Arg Gln Pro Ala Pro Asp Asp Glu Thr Ile Cys
                165                 170                 175

Gln Ile Asn Glu Glu Pro Gly Tyr Val Leu Phe Ser Ala Leu Gly Ser
            180                 185                 190

Phe Tyr Val Pro Leu Thr Ile Ile Leu Ala Met Tyr Cys Arg Val Tyr
        195                 200                 205

Val Val Ala Lys Arg Glu Ser Arg Gly Leu Lys Ser Gly Leu Lys Thr
    210                 215                 220

Asp Lys Ser Asp Ser Glu Gln Val Thr Leu Arg Ile His Arg Lys Asn
225                 230                 235                 240

Ala Pro Ala Gly Gly Ser Gly Val Ala Ser Ala Lys Asn Lys Thr His
                245                 250                 255

Phe Ser Val Arg Leu Leu Lys Phe Ser Arg Glu Lys Lys Ala Ala Lys
            260                 265                 270

Thr Leu Gly Ile Val Val Gly Cys Phe Val Leu Cys Trp Leu Pro Phe
        275                 280                 285

Phe Leu Val Met Pro Ile Gly Ser Phe Phe Pro Asp Phe Lys Pro Pro
    290                 295                 300

Glu Thr Val Phe Lys Ile Val Phe Trp Leu Gly Tyr Leu Asn Ser Cys
305                 310                 315                 320

Ile Asn Pro Ile Ile Tyr Pro Cys Ser Ser Gln Glu Phe Lys Lys Ala
                325                 330                 335

Phe Gln Asn Val Leu Lys Ile Gln Cys Leu Arg Arg Lys Gln Ser Ser
            340                 345                 350

Lys His Ala Leu Gly Tyr Thr Leu His Ala Pro Ser Gln Ala Leu Glu
        355                 360                 365

Gly Gln His Lys Asp Met Val Arg Ile Pro Val Gly Ser Gly Glu Thr
    370                 375                 380

Phe Tyr Lys Ile Ser Lys Thr Asp Gly Val Cys Glu Trp Lys Phe Phe
385                 390                 395                 400

Ser Ser Met Pro Arg Gly Ser Ala Arg Ile Thr Val Pro Lys Asp Gln
                405                 410                 415
```

Ser Ala Cys Thr Thr Ala Arg Val Arg Ser Lys Ser Phe Leu Gln Val
420 425 430

Cys Cys Cys Val Gly Pro Ser Thr Pro Asn Pro Gly Glu Asn His Gln
435 440 445

Val Pro Thr Ile Lys Ile His Thr Ile Ser Leu Ser Glu Asn Gly Glu
450 455 460

Glu Val
465

<210> 5
<211> 1194
<212> DNA
<213> Homo sapiens

<220>
<221> promoter
<222> (1)..(1076)

<220>
<221> misc_feature
<222> (1077)..(1079)
<223> start codon

<400> 5
ggagagacag aaaccacacg gagagacaca gagacaagag ggtccgagaa agacagagca 60
agcgcacacg tgccagtgag agacagaagg agacacccag agaacccgag ggagacgcag 120
aaacaagaga gttggagaga cacggagacg ggcagagaca cagcgaacga cattgaacag 180
gtgcgctagg gacccacaaa gatgaactgc tggggaaata gactgagaga gaaatggaga 240
gacaagagag cactagggaa agacggagac acacatgaac tagcgagcga cacagtgaca 300
ggcagagaca cagagagaca gaatggatca gaaagagact gagggagaga cgggcaagac 360
tgtgagaaag atagggagcg agagcgcaag ccctgcagag aaagtgcgct agcgaaagag 420
acacacacag agagactgag tgacaaacag agtggaagag ataaatgaga gtaacaggaa 480
agagacagcg tgcacgtgct atcgagagac ggagaaacag agacagacat aagtgagaga 540
caaggatgag aaagaatcag gaaacggtga gacacataca gacacacaac ccgagagata 600
aagaaaaaaa gtgcgctagc gagagacact ggagaagaga cagaaactga cggtcgtaaa 660
cagacagaaa cagagagcgc acgcgcatgc gctagcgcta gcgaccaaaa ctccccggag 720
ccagagacag tgtgagagac aagcagagaa agcgcacgca cgcacgcgcc agcaggagaa 780
acagatgaga ggaaatcaga gccctggaga gagacaggca gacagatctg gagagtccgg 840
aaaggagcca tagaagctgc ccgcactggg gatggagccg tgcggaaacc cggggtaggg 900
ggtcctgcag cgtccttgct gggcgcggag gcttctcccc ttgacgggtg actaactctg 960
cctgcgtgtt tcttttgtca ccagcatagg cactgagtgc ggtctgtgca ccccttgcc 1020
acccaccggt gccggcactg agcctgcaac ctgtctcacg ccctctggct gttgccatga 1080
cgtccacctg caccaacagc acgcgcgaga gtaacagcag ccacacgtgc atgcccctct 1140
ccaaaatgcc catcagcctg gcccacggca tcatccgctc aaccgtgctg gtta 1194